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16. Abstract
The kinetics and characteristics of phosphate-limited algal cultures were studied on a laboratory scale in order to : (1) evaluate kinetic parameters of algal growth in relation to phosphorus concentration; (2) derive an expression describing the growth rate-limiting nutrient relationship; and (3) determine desirable or optimum design and operating parameters for effective phosphorus removal by algal systems. Phosphorus limiting algal growth was described by ~~$\mu = \frac{\hat{\mu} S_l}{k_s + S_l}$, where μ = specific growth rate,~~ the Monod growth model for specific growth rate. ~~$\hat{\mu}$ = maximum specific growth rate, S_l = concentration of limiting nutrient, and k_s = a saturation constant numerically equal to the nutrient concentration at which the specific growth rate is one-half the maximum value.~~ Maximum specific growth rate for the algae tested was 4.19/day, and all growth characteristics were significantly affected by variations in pH and temperature of the culture. Removals of moderate concentrations of phosphorus (≦7 mg/l as P) in excess of 85% can be accomplished by continuous algal cultures. (Lowry-Texas)

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KINETICS OF ALGAL SYSTEMS IN WASTE TREATMENT

Phosphorus As A Growth-Limiting Factor

Final Report - Part I

by

Mario D. Zabat
William J. Oswald
Clarence G. Golueke
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Federal Water Quality Administration
U. S. Department of the Interior
Grant No. 17010-DZQ

September 1970

Sanitary Engineering Research Laboratory
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CHAPTER I

INTRODUCTION

One of the major problems in water conservation and pollution control in the United States today is the excessive enrichment of surface waters by plant nutrient minerals. Uncontrolled enrichment is transforming many surface water bodies into unaesthetic, less useful, and more expensive-to-develop water resources.

The adverse effects of excessive inorganic enrichment of surface waters are well known and are well documented. The most significant is probably the hastening of the natural process of aging, or eutrophication, of lakes, reservoirs, and other relatively stagnant waters as a result of which the useful lives of these water bodies can be markedly reduced. More commonly recognized objectionable results of inorganic surface water enrichment are algal blooms and overabundant growths of undesirable or less desirable aquatic life. These, in turn, affect the water quality in many ways and interfere with many existing and potential beneficial water uses.

Of the various known or available methods for controlling excessive surface water enrichment and the associated problems, it is presently generally agreed that limitation of the phosphorus input into receiving waters through the elimination of this nutrient element from sewage and other waste waters offers the most practical, effective, and widely applicable approach. The best of conventional waste treatment, however, removes phosphorus only to a small extent. Thus, there is a need for improved or supplementary waste treatment processes that are capable of removing phosphorus effectively.

Among the possibilities for removing large amounts of phosphorus, as well as other plant nutrients, from waste waters is through biological removal by algal cultures or systems. Algal systems have been successfully used for years in the treatment of organic wastes from communities and various industries. However, relatively little is known concerning their application for phosphorus removal. This study was undertaken to obtain some information that may be of aid in the improvement or development of algal systems for phosphorus control from waste discharges.

OBJECTIVES

The objectives of the study may be stated as follows:

1. To study the kinetics and characteristics of phosphate-limited algal cultures.
2. To evaluate the kinetic parameters of algal growth in relation to phosphorus concentration.
3. To determine a suitable expression that can describe the growth rate-limiting nutrient relationship.
4. To determine desirable or optimum design and operating parameters for effective phosphorus removal by algal systems.

PREVIOUS INVESTIGATIONS BY OTHERS

Studies on the use of algal systems for phosphorus removal have been relatively few. These studies include laboratory and pilot-plant scale investigations as well as evaluations of full scale installations.

Oswald et al. (1) reported an investigation at Concord, Calif. to reclaim both water and algae from domestic sewage. A plastic-lined experimental pond was used which was operated at a depth of 5-12 inches and a detention period of 2-4 days. Evaluation of the effects of mixing on algal production and pond performance was included in the study and they showed that adequate mixing was essential and definitely improves pond performance and the production of algae. Analysis of the liquid phase of the pond culture showed 50-57 per cent removal by the pond of the phosphorus in the settled sewage of the city of Concord. When the pond culture was flocculated with alum and subsequently settled, phosphorus removals of 92-96 per cent were obtained. The results from this study led to the development of a "high-rate" pond treatment process and a 2/3-acre high-rate pond facility at the Sanitary Engineering Research Laboratory of the University of California at Richmond, Calif. The latter was equipped with propeller pumps for mixing, was asphalt-lined, and can be operated at a depth of 2-16 inches and a detention period of 1-3 days. In studies with this pond (2), using settled Richmond City, Calif. sewage, they found average phosphorus removals by the pond as high as 85 per cent.

Witt and Borchardt (3) studied the effects of temperature, light intensity, and culture density on the rate of growth and rate of removal of nutrients from sewage effluent of mixed cultures of Scenedesmus and Chlorella with a turbidostat-type of laboratory culture apparatus. They found that there was an optimum level of cell concentration that produced the most effective removal of nutrients. The best result at 20°C. was obtained with a concentration of 9×10^6 cells per ml which absorbed phosphorus at a rate of 0.28 mg P/hr. Phosphorus removal was found to be more effective at 25°C. than at 20°C. They reported that the removal of phosphorus was never complete but it continued long after the absorption of nitrogen had ceased, suggesting that this nutrient was being stored by the cells.

In another water reclamation study, Bush et al. (4) used a U-shaped algal pond to evaluate the effectiveness of algae for removing dissolved solids from activated sludge effluent at detention periods of 29, 14.5, 7 and 2.5 hrs. The phosphorus concentration of the influent to the pond varied from 1.6 to 4.8 mg P/1. The pond was continuously supplied with supplemental carbon dioxide to maintain the pH at optimum levels for algal growth. They found the pond to be able to remove from 19 to 68 per cent of the phosphorus in the influent, with the higher removals obtained at the shorter detention periods.

In controlled field studies of the performance of raw sewage ponds at Fayette, Mo., Neel et al. (5) obtained phosphorus reductions ranging from 83 to 92 percent by ponds operated at a depth of 2.5 ft and loadings of 20, 40, 60, 80, and 100 lbs BOD/acre/day. The ponds treated raw municipal wastes that included wastes from small industries, slaughter-houses, and locker rooms. The organic phosphorus in the raw sewage ranged from 4.4 to 18 mg/1 and orthophosphate from 16 to 39.8 mg/1 as PO_4^{-3}. No particular relationship of phosphorus removal to loading nor pronounced seasonal differences in removal were observed.

Mackenthun and McNabb (6) also observed significant phosphorus reductions at 3 stabilization pond installations in Wisconsin but under the climatic conditions of the region, there was considerable variation in the removals during the year. The performance of the ponds was studied by regular sampling once a month during 24-hr periods of normal operation. Phosphorus concentrations in the ponds (or effluents) were found to be lowest during the summer and fall and highest in the winter when the ponds were under ice cover. Phosphorus reductions during the summer ranged from 58 to 80 per cent or higher depending on the pond loadings, which ranged from a low of 3.5 lbs BOD/acre/day to a high of 307 lbs BOD/acre/day.

Bogan et al. (7) studied nutrient removal from secondary effluent by algal photosynthesis in the laboratory and in the field on a pilot plant-scale. In the laboratory, they found that phosphorus could be removed both by metabolic uptake by the algae and by chemical precipitation and absorption resulting from increase in the pH due to photosynthetic activity. Based on the laboratory results, a high rate tertiary stage treatment process for phosphorus removal employing an illuminated contact basin and a cell separator was developed and tested in the laboratory and in the field on a pilot-plant scale. The process was found to be functionally feasible under controlled laboratory conditions with sufficient light. In the field, however, difficulty in achieving adequate light intensities in the contact tank, because of the large depth of the culture, markedly inhibited the rate of pH change and the process was therefore subsequently modified to include both the use of lime and the effect of natural illumination.

Hemens and Mason (8) reported a 10-month study of nutrient removal by algal photosynthesis and pH increase using a 10,000 ft long shallow (4 in. working depth) algal stream and Pretoria, South Africa, biofilter effluent. The effluent, which had an average phosphorus concentration of 8.3 mg P/1, was passed through the algal pond at a rate to obtain a detention period of approximately 24 hrs. It was found that more than 90 per cent of the nitrogen and phosphorus could be removed by the pond during the warmer months. The removal of phosphorus was mainly due to precipitation of calcium phosphate and ammonia was lost to the atmosphere as a result of increase in the pH caused by algal photosynthetic activity. The removal efficiencies were, however, markedly less during the winter when the pH values reached were not as high as those in the summer.

In a later paper, Hemens (9) described a modified nutrient removal system designed to overcome several shortcomings of the shallow algal stream. The modified system consists of a number of half-submerged paralled disks rotating in a tank and the disks were covered with algae growth. In preliminary studies, he found that, as in the shallow stream system, precipitation of calcium phosphate occurred when the pH reached high levels.

Reid and Assenso (10, 11) investigated the feasibility of using attached algae to reduce the nutrient content of sewage effluent and reported that these algae can be easily grown on shallow water depth surfaces or on moving surfaces and can substantially remove nitrogen and phosphorus from the sewage. The attached algae could be easily harvested by scraping.

In another study, Assenso and Reid (12) determined the removal of nitrogen and phosphorus in seven bio-oxidation ponds in central Oklahoma and reported that the ponds removed 30 to 95 per cent of the nitrogen and phosphorus. The pond loadings ranged from 13 to 150 lbs BOD/acre/day. Using the surface response technique in the analysis of the data, the BOD, nitrogen, and phosphorus loadings for optimum removals were found to be 52, 12, and 1.6 lbs/acre/day, respectively.

Loehr and Stephenson (13) evaluated a 6.2-acre oxidation pond in Johnson Co., Kansas, as a tertiary treatment device. The pond received trickling filter effluent and provided a detention period of 1.8 to 3.3 days. They found that total and soluble phosphorus removal by the pond occurred only during the summer months and after the temperature increased in the spring, and concluded that a normal oxidation pond is not an effective tertiary device to control the nutrients discharged from waste treatment facilities.

Studies on the use of oxidation ponds for the tertiary treatment of municipal wastes have also been reported by Weiss (14). Under the extremely high BOD loadings used, which ranged from about 300 to 700 lbs BOD/acre/day, the ponds proved to be ineffective in removing phosphorus from the treatment plant effluent.

Buzzell and Sawyer (15) treated lime-treated raw sewage, after recarbonation and without recarbonation, in laboratory model stabilization ponds and found that 75-80 per cent of the phosphorus remaining after lime treatment could be removed. The lime treatment removed 80-90 per cent of the total phosphorus and more than 97 per cent of the soluble inorganic phosphorus in the raw sewage. Treatment of the lime-treated sewage in laboratory activated sludge units was unsuccessful because of the low BOD of the waste.

Gates and Borchardt (16) studied the rates of growth and nutrient extraction in sewage effluent of Oscillatoria cultures with a constant-density culturing apparatus (turbidostat) and reported that the growth rate is a function of the total inorganic nitrogen concentration in the influent when the growth rate was severely limited by this nutrient. A similar relationship, in general, was found for phosphorus. Two different culture densities and two different incident light intensities were used in their studies.

In another laboratory study, Borchardt and Azad (17) investigated the uptake of phosphorus by uni-algal and mixed cultures of Chlorella and Scenedesmus with a similar turbidostat culture device. With the culturing conditions that they used, they found the growth rate of the algae to be independent of the phosphorus concentration in the substrate as long as this was above 3 per cent (as PO_4^{-3}) of the dry weight of the cells. Below this level, which was designated as the critical phosphate concentration, the growth was proportional to the phosphate concentration. When the phosphorus in the substrate was between 3 and 9 per cent (as PO_4^{-3}) of the cell dry weight, all the phosphorus was assimilated by the algae. When phosphorus was supplied in excess of 9 per cent of the dry weight of the cells, the excess remained in solution. Phosphorus-starved cultures were found to be able to absorb phosphates rapidly upon exposure to phosphorus-rich substrates (18).

Davis and Wilcomb (19, 20) studied the rates of hydrolysis and assimilation of three inorganic condensed phosphates by pure cultures of green and blue green algae. The studies were made under both static (batch) conditions involving 30 days' duration in Erlenmeyer flasks and flow-through conditions involving 1.5 days' duration using 4-ft long horizontal tubes. The condensed phosphates were sodium tripolyphosphate, tetrapotassium pyrophosphate, and sodium hexametaphosphate. For comparison and control purposes, the hydrolysis rates of the condensed phosphates in raw domestic sewage and in sterile synthetic algal nutrient medium, and algal growth and phosphorus uptake rates in nutrient medium alone were also determined. They found that the fastest rate of hydrolysis occurred in the raw domestic sewage and the slowest in the sterile algal nutrient medium. As might be expected, the hydrolysis and phosphorus assimilation rates for the different condensed phosphate forms varied among the algal genera and species. The rates were generally greater for sodium hexametaphosphate than for the other two forms. With few exceptions, the hydrolysis and phosphorus uptake rates were higher under the flow-through conditions than under the static conditions.

CHAPTER II

LITERATURE REVIEW

EUTROPHICATION OF SURFACE WATERS

Eutrophication, a natural phenomenon in all surface waters, has become of great concern in the fields of sanitary engineering, limnology, and water resources development. This is evidenced by the increased amount of activity in this area. Discussions and reviews of the basic concepts and problems of eutrophication have been frequent in recent years (21-33).

Various meanings and definitions have been given to eutrophication. A widely accepted concept, however, is that it is the process of aging leading to extinction of lakes and reservoirs. The aging process occurs in all natural waters but is more pronounced in such water bodies as lakes and reservoirs which have relatively little movement. A young lake is a relatively barren or sterile body of water in terms of the amount of biological life that it can support. This stage is known as an oligotrophic, or infertile, stage. As the lake ages, it is continually enriched with nutritive materials from its drainage basin. This results in the production of more and more phytoplankton as well as higher forms of aquatic life so that, in time, the lake becomes fertile or eutrophic. As aging progresses, deposits from the drainage basin plus those resulting from biological activity that are retained by the lake accumulate to the extent that rooted aquatic plants take over and gradually convert the lake to marshes or moors and eventually to dry land.

Eutrophication of a lake or reservoir will occur naturally but the normal process is so slow that it takes hundreds to thousands of years for a lake to become eutrophic. The process, however, can be accelerated by man through his activities, such as alteration of the landscape, urbanization, agricultural activities, and the discharge of urban and industrial waste waters, which increase the rate of supply of nutritive materials to the water body.

A classical and well-publicized example of accelerated lake eutrophication which has resulted from artificial fertilization has been described by Hasler (34) in the case of the Zurichsee, a lake in the foothills of the Alps. The lake is composed of two distinct basins, the Obersee and the Untersee, separated only by a narrow passage. Since shortly before the turn of the century, the Untersee, which is the deeper of the two basins, has received the urban drainage from a group of small communities with a total population of about 110,000 and became strongly eutrophic within only a few decades. The shallower basin, the Obersee, received no major urban drainage and has retained its oligotrophic characteristics. Thirty-six other lakes in Europe and the United States in which oligotrophy changed to strong eutrophy within a short period due to urban effluents were also described by Hasler. In the United States, a well-publicized case is that of the Yahara Lake Chain at Madison, Wisconsin (35). Other lakes which have become artificially eutrophied are Lake Washington at Seattle (36, 37), Lake Zoar in Connecticut (38), and Lake Erie (39).

The aging process of a lake has been depicted by Hasler in the graph shown in Figure 1. The solid line in the figure represents the course of natural eutrophication. The broken lines show the effect of fertilization on the aging process in (a) the oligotrophic stage, and (b) in the eutrophic stage. According to Hasler, reducing conditions at the bottom of eutrophic lakes also accelerate the rate of eutrophication by releasing more nutrients into the water for recirculation than under oligotrophic conditions, and may be one of the reasons for the steep rise in the curve of eutrophy.

Control Measures

Various methods have been suggested and tried to control artificially-induced eutrophication and excessive aquatic growths in surface waters. The methods include land use control, diversion of effluents, dredging, mass harvesting of fish and/or aquatic plants, chemical poisoning, aeration of the hypolimnion of deep lakes, drawing off hypolimnetic waters, and nutrient removal from waste waters. Many of the methods are not very practical and some have only limited application.

Land use control and regulation such as zoning of residential, industrial, recreational, and natural areas, and good farming practices are recognized sound measures that can substantially reduce the flow of sediments and nutrients into water bodies.

One recourse for lakes in which urban effluents are the principal source of nutrients is to divert the effluents around the lake to its outlet or to another watershed. Hasler (34), Edmonson (40), and Thomas (25) have reviewed a number of such practices. The

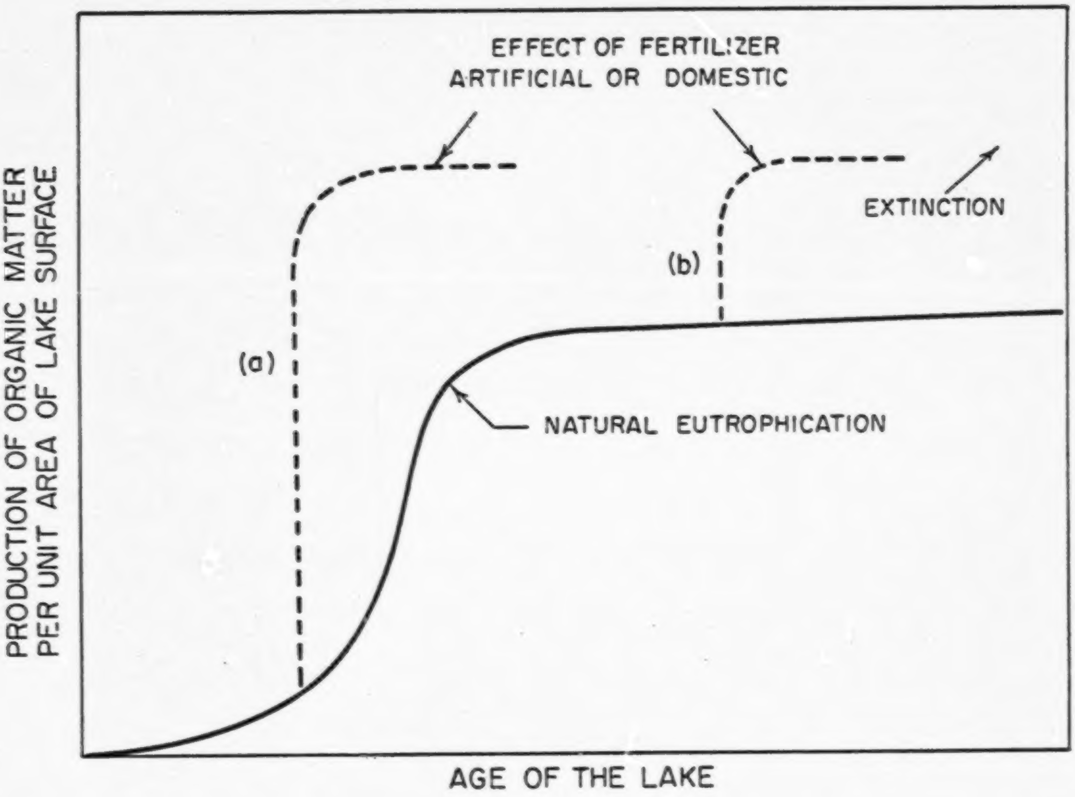

FIGURE I. HYPOTHETICAL CURVE OF EUTROPHICATION (34)

method has been proposed for or practiced at Lakes Kegonsa and Waubesa in Wisconsin (41), Lake Washington at Seattle (42), and Lake Tahoe in California (43). It is expedient but usually is expensive and may only transfer the problem to some other place.

Substantial amounts of nutrients are always present in the sediments and therefore, dredging has been considered to remove this concentrated source of nutrients. Stewart and Rohlich (31), however, have pointed out that dredging may have little value if the concentrations of nutrients, particularly phosphorus, in the overlying water are maintained at relatively high levels by the inflowing waters. Furthermore, dredging may have doubtful value in improving the quality of the water since the sediments below those removed could be as rich and contribute just as significantly as those removed. Moreover, the process of dredging itself mechanically releases soluble phosphates.

Mass harvesting or removal of coarse fish, aquatic weeds, and algae has been suggested to reduce the amount of nutrients in a body of water (31). However, this method could be difficult, laborious, time-consuming, and expensive and, although several tons of biological material may be removed from a body of water, the total amount of nutrients removed may not be significant in relation to those present or supplied to the water. It may, however, help to alleviate the problems caused by aquatic nuisances and may be a suitable measure in cases where the major sources of nutrients are difficult to control. In this method as well as in dredging, disposal of the materials removed could present a difficult problem.

Augmentation of the flow into a lake with water low in nutrients, which will not only dilute the water and nutrients but will also increase the rate of flushing and thereby reduce the detention time, is another method for reducing the concentration of nutrients in a lake. This approach, among others, has been suggested for the problem of the Madison, Wisconsin lakes by Schroepfer et al. (31). The method was also recommended by Sylvester and Anderson (46) for Green Lake, a small eutrophic lake near Seattle, using Seattle city water. The method is currently being evaluated in Green Lake (42) and in Lake Bled in Yugoslavia (47).

In deep eutrophic lakes that become stratified thermally, plant nutrients usually accumulate in the hypolimnion during the summer stratification period. Drawing off hypolimnetic waters to the lake's outlet during this period will remove part of the nutrients in the lake (25). A possible consequence of this operation is the impairment of the quality and waste assimilation capacity of downstream waters (48).

Attempts have also been made in Europe and the United States to aerate the hypolimnion or mix it with the epilimnion with compressed air (25, 49). By these methods, homoiothermal conditions, isometric concentrations of phosphorus, and increases in dissolved oxygen can be achieved. Application of these methods would, however, be practical only for small and relatively shallow lakes and impoundments as they could be rather expensive for lakes which are large and deep.

An alternative to effluent diversion and to most of the other methods previously discussed is the elimination of nutrients from waste waters entering the lake. Waste waters have been shown to have often been responsible for the early and accelerated eutrophication of surface waters, therefore, nutrient removal would be of general and wide applicability. In this approach, it is not necessary to remove all the nutrients present in waste waters but only one (or two) essential nutrient so that the concentration of this nutrient in the receiving water will be growth-limiting. At present, nitrogen and phosphorus are generally agreed to be the critical nutrients because they are usually available in nature in limited quantities in relation to other essential nutrients and thus are often the nutrients that limit and control the biological productivity of both land and water areas. From his studies of 17 lakes in southeastern Wisconsin, Sawyer (50) concluded that if concentrations of inorganic nitrogen and phosphorus of 0.30 mg/1 and 0.015 mg/1, respectively, were not exceeded in any of these lakes at the start of the active growing season (time of spring overturn in northern climates), nuisance-level algal blooms would not be expected to follow.

At present, phosphorus appears to be the best single nutrient to employ as a limiting factor because certain blue-green algae can fix nitrogen from the atmosphere and thus algal nuisances can also develop even in the absence of significant amounts of nitrogen as long as the supply of phosphorus is abundant. In the Zurich area, for example, Thomas (25) reported that in the test areas known to him, phosphate is the most suitable for removal for the following reasons: (a) phosphate is present only in small quantities in oligotrophic lakes in the Zurich area, (b) the natural tributaries running into these lakes contain very little phosphate as long as they are not subjected to human pollution, but contain large quantities of nitrate, (c) rain water often contains large quantities of nitrogen compounds that can be utilized by plants, (d) bacteria and blue-green algae living in the water are, under certain conditions, able to combine gaseous nitrogen organically, (e) a high percentage of the nitrogen compounds from the putrefying parts of organisms return to the cycle of substances as phosphorus compounds, and (f) phosphorus can be more readily eliminated from sewage than nitrogen compounds.

During the past decade, considerable attention has been given to the study and development of processes for phosphorus removal from waste waters. Work done in this area is briefly summarized in another section.

FORMS AND MEASUREMENT OF PHOSPHORUS

The forms and measurement of phosphorus in waters and waste waters are important from a treatment and control standpoint. Phosphorus may be present in waters and waste waters in various forms and compounds. It may be present as particulate (precipitate or adsorbed) compounds or in soluble form. The sum of the particulate and soluble phosphorus in a water or waste water is the total phosphorus. The total phosphorus is determined by digesting a whole (unfiltered) sample and analyzing for the phosphorus present. If a filtered sample is digested and analyzed, the phosphorus determined is the soluble phosphorus. The particulate phosphorus is determined as the difference between the total and soluble phosphorus.

Both inorganic and organic compounds or ions may be present in the particulate and soluble forms of phosphorus. Each of these, in turn, may be broken down into "orthophosphates" and "condensed phosphates." In water and waste water analysis, it is common and often sufficient to divide the phosphorus (particulate, soluble, total) into: (1) inorganic orthophosphates, (2) condensed phosphates, and (3) organic phosphates. The condensed phosphates, as determined from an analysis, may included some organic phosphates.

The particulate form of phosphorus is not immediately available for phytoplankton growth and metabolism, but all phosphorus should be considered potentially available. The total phosphorus and total soluble phosphorus determinations are generally sufficient for most purposes.

In a broad sense, orthophosphates include orthophosphoric acid, H_3PO_4, orthophosphate compounds, and the orthophosphate ions. An excellent treatment of the chemistry of phosphorus and its compounds has been given in a recent work by Van Wazer (51) and should be consulted for details and information on the subject.

Orthophosphoric acid is a tribasic acid which is quite strong with respect to the first dissociation constant, moderately weak with respect to the second, and very weak with respect to the third. The acid ionizes as follows:

$$H_3PO_4 \rightleftharpoons H^+ + H_2PO_4^-$$

The first dissociation constant, K_1, in the temperature range of 0-60°C. is given by

$$-\log K_1 = 799.31/T - 4.5535 + 0.01386T$$

where T is the absolute temperature (°K.). At 25°C., this leads to a value of K_1 of 7.107×10^{-3}, and a value of pK_1 of 2.148.

The second ionization is

$$H_2PO_4^- \rightleftharpoons H^+ + HPO_4^{--}$$

and the second dissociation constant, K_2, is given by the following equation:

$$-\log K_2 = 2073.0/T - 5.9884 + 0.02912T$$

The above equation is correct for the temperature range of 0-50°C. At 25°C., $K_2 = 7.99 \times 10^{-8}$, and $pK_2 = 7.098$.

The third ionization is

$$HPO_4^{--} \rightleftharpoons H^+ + PO_4^{---}$$

At 25°C., K_3 is 4.8×10^{-3} and $pK_3 = 12.319$.

From the above relationships, it can be seen that in dilute solutions less than 1 per cent of the HPO_4^{--} present is ionized at pH values less than 10.32, less than 1 per cent of the $H_2PO_4^-$ is ionized at pH values less than 5.09, and more than 99 per cent of the H_3PO_4 is ionized at pH values greater than 4.15. Thus, in waters in the normal range of pH values, the phosphate present would consist of both $H_2PO_4^-$ and HPO_4^{--}.

The ions, H_2PO^-, HPO_4^{--}, and PO_4^{---}, are orthophosphate ions. Commonly known orthophosphate compounds are those of sodium, potassium, calcium, and ammonia. The orthophosphates of the alkali metals, ammonia and the lower molecular weight substituted ammonium ions, are all quite soluble in water, except for trilithium orthophosphate.

On the other hand, the orthophosphates of the multiply charged or heavy metals (Ca, Fe, Al) are all relatively insoluble. Large quantities of the inorganic forms of phosphorus present in soils occur as insoluble calcium, iron or aluminum phosphates (52). The calcium salts predominate in neutral or alkaline conditions, the iron and aluminum salts in acid surroundings. Phosphorus may be transported to surface waters from soils or land by runoff and/or drainage waters in the simple orthophosphate form, as soluble hydrolyzable (condensed) phosphates, or adsorbed on soil particles.

The condensed phosphates are polymers of interconnected PO_4's and may be chains, rings, or branched polymers. They can probably be thought of as those phosphate compounds or ions which are not orthophosphates.

An old notation for phosphate compounds, which has led to considerable confusion in the past but still persists today, divides all phosphates into orthophosphates, pyrophosphates, and metaphosphates. This notation was based on the old "double oxide" notation of Berzelius, in which salts of the oxyacids are written in terms of their oxides. Thus

Orthophosphoric acid, $3H_2O \cdot P_2O_5 \cong H_3PO_4$

Pyrophosphoric acid, $2H_2O \cdot P_2O_5 \cong H_4P_2O_7$

Metaphosphoric acid, $H_2O \cdot P_2O_5 \cong HPO_3$

Later, the concept of formation of polyphosphoric acids (polyphosphates) from ordinary phosphoric acid by abstraction of water was introduced and the following series was set up:

Orthophosphoric acid, H_3PO_4 = H_3PO_4

Pyrophosphoric acid, $H_4P_2O_7$ = $2H_3PO_4$ less H_2O

Triphosphoric acid, $H_5P_3O_{10}$ = $3H_3PO_4$ less $2H_2O$

Tetraphosphoric acid, $H_6P_4O_{13}$ = $4H_3PO_4$ less $3H_2O$

Etc.

The general equation of the above series is $mH_3PO_4 - (m - 1)H_2O$. When m approaches infinity, the formula becomes $(H_3PO_4 - H_2O)_m$, which is the metaphosphoric acid of the old notation.

The term "condensed phosphates" now covers the pyrophosphates, metaphosphates, polyphosphates, and another group recently introduced known as the ultraphosphates. It is a generic title for all phosphates, the acids of which have less water than orthophosphoric acid, $3H_2O \cdot P_2O_5$.

Common inorganic condensed chain phosphates are tetrasodium pyrophosphate ($Na_4P_2O_7$), tetrapotassium pyrophosphate ($K_4P_2O_7$), sodium tripolyphosphate ($Na_5P_3O_{10}$), and various vitreous (glassy) sodium phosphates which have been and are called the misnomer sodium "hexametaphosphate." These compounds are extensively employed for building detergents and water softening applications. A number of organic condensed phosphates are present in living organisms where they perform important biological functions.

Condensed phosphates hydrolyze to the uncondensed (ortho) form and are thus often termed "hydrolyzable" phosphates.

In the hydrosphere, phosphorus is normally present in only small quantities and occurs almost exclusively as phosphates. The phosphates may exist as soluble inorganic phosphates, insoluble ferric and calcium phosphates, soluble and colloidal organic phosphates, and particulate organic phosphates. In lakes, the soluble inorganic phosphate is usually around 10 per cent of the total phosphorus, about half of the total is organic, and the rest particulate inorganic phosphate (53).

SOURCES OF PHOSPHORUS TO SURFACE WATERS

In studies for the control of excessive enrichment of surface waters, the sources of nutrients and the amounts and forms of nutrients contributed by each source are paramount consideration. As indicated previously, nutrients can enter surface waters naturally or through the activities of man, but the latter is generally a more significant cause of premature surface water eutrophication. The major natural sources of nutrients recognized to date are (1) air, (2) rainfall, (3) water plants and animals, (4) muds under water bodies, and (5) runoff and drainage from uncultivated and forest lands. The air does not appear to be a significant source of phosphorus to surface waters although there is at least one potential gaseous source, namely phosphine gas, PH_3. Man-generated sources are (1) domestic sewage, (2) industrial wastes, (3) municipal and industrial water conditioning, (4) urban runoff and drainage, and (5) runoff and drainage from cultivated land and from land on which animals are raised.

Relatively few studies have been made in the United States to determine the amount of nutrient materials contributed by individual sources. The data from these studies have been used to estimate the total nationwide contribution from each source, such as shown in Table I, II, and III for phosphorus (54). From these data, it can be seen (Table II) that surface waters of the nation receive 0.9-1.7 billion pounds of phosphorus annually from natural and man-generated sources. As much as 75 per cent of the phosphorus apparently come from man-generated sources (Table II) and roughly half of this phosphorus comes from domestic sewage (Table III).

The phosphorus in domestic sewage originates from the following sources: (1) human wastes -- feces, urine, and food wastes disposed through garbage grinders, (2) built detergents, (3) water supply, and (4) infiltration waters. The majority normally comes from human wastes and detergents. The contribution by human wastes may be estimated from per capita dietary phosphorus requirements, daily per capita phosphorus excretion, and the daily per capita phosphorus contribution to waste waters.

The amount of phosphorus ingested under ordinary conditions through food by an adult varies from 1.2 to 2.0 grams per day, or 0.97 to 1.6 lb P per year (55). A recent report of Task Group 2610 P of the American Water Works Association (29) cited reported figures from 150 U.S. dietaries as follows: maximum -- 2.3 lb P per capita per year, minimum -- 0.5 lb P per capita per year, and mean -- 1.3 lb P per capita per year. Assuming that there is little accumulation of phosphorus in the mature human body, the excretion of phosphorus should equal its consumption as stated above. The same report cited a reported figure of the phosphorus excretion in feces and urine of about 1.4 lb P per capita per year, which lends some support to the above assumption.

TABLE I

APPROXIMATE QUANTITIES OF PHOSPHORUS FROM SOURCES EITHER IN OR ENTERING U.S. SURFACE WATERS (54)

Source	Quantities 1,000,000 lb/yr
NATURAL	
Rainfall (direct into surface water)	2-17
Aquatic plants	0-107
Waterfowl, fish, bottom fauna, and the like	n.a.
Muds under water bodies	n.a.
Runoff from	
Forest land (including commercial forests)	243-587
Other land	n.a.
MAN-GENERATED WASTE WATER	
Domestic	
Human and food wastes	137-166*
Washing wastes	250-280*
Industrial	n.a.
RUNOFF FROM	
Urban land	19
Cultivated land	
Fertilized	130-380
Unfertilized	n.a.
Land on which animals are kept	170

n.a. Not Available

*Data based on the assumption of 30% removal of phosphorus in treatment plant

TABLE II

NATURAL AND MAN-GENERATED SOURCES OF PHOSPHORUS (54)

Source	Phosphorus 1,000,000 lb/yr	Percent
Natural	245-711	26-41
Man-generated	686-1,015	74-57
Total	931-1,726	100

TABLE III

MAN-GENERATED SOURCES OF PHOSPHORUS (54)

Source	Phosphorus 1,000,000 lb/yr	Percent
Domestic sewage	387-446	56-44
Runoff from		
Urban land	19	3-2
Cultivated land	110-380	16-37
Land on which animals are kept	170	25-17
Total	686-1,015	100

The amount and fraction of the phosphorus in domestic sewage contributed by detergents have been estimated by a number of investigators. Sawyer (56) estimated the detergent contribution to be about 1.6 lb P per capita per year for 1950. An estimate of 1.9 lb P per capita per year was made by Engelbrecht and Morgan (57) for 1955. Task Group 2610 P of the American Water Works Association (29) estimated the detergent contribution to be 2.1 lb P per capita per year, based on the consumption for 1958 of phosphorus for built detergents, and concluded that the contribution of detergent-derived phosphorus to domestic wastes could equal or exceed that from other sources. In 1965, Sawyer (58) stated that the phosphorus from detergents represented 50-75 per cent of the total phosphorus concentration in domestic waste water. As early as 1952, the same worker (56) estimated the phosphorus concentration of domestic sewage to have doubled within the previous six-year period, as a result of the increased use of synthetic detergents. In a recent study (1964-65) of the per capita phosphorus contribution in the domestic sewage of a small residential community in California, San Ramon, Jenkins (59) determined that 60 per cent of the total phosphorus in the domestic sewage originated from synthetic detergents.

Certain industrial waste waters, such as slaughterhouse and meat packing wastes, are known to contain significant quantities of nitrogen and phosphorus while others have practically none of these nutrients. The significance of industrial contributions would depend on the nature, location, and size of the industry. At the present time, very little is known about the phosphorus concentrations and contributions of industrial wastes.

A part of the phosphorus present in a sewage or industrial waste may have its origin from the phosphorus compounds used in water conditioning or treatment. Phosphorus compounds are used in water treatment for (a) water softening, (b) sequestration or the complexing of hardness-causing cations in water to prevent the formation of precipitates, (c) threshold treatment, and (d) corrosion control. The phosphorus compounds commonly employed are trisodium phosphate (Na_3PO_4), tetrasodium pyrophosphate ($Na_4P_2O_7$), tetrapotassium pyrophosphate ($K_4P_2O_7$), sodium "hexametaphosphate", and various other metal-containing glassy phosphates especially those of calcium, magnesium or zinc.

Task Group 2610P (29) stated in 1966 that compounds containing nitrogen or phosphorus were employed then in less than 30 per cent of the municipal water in the United States but that their use is steadily rising and is predicted to reach 50 per cent in 1985. Phosphate compounds, however, are widely used in industrial water treatment.

The contribution of runoff and drainage from agricultural land to the nutrients to surface waters has been estimated by some workers. Sawyer (50) reported an average phosphorus contribution from agricultural land in the Madison, Wisconsin area of 0.4 lb P per acre per day. In extensive studies of different agricultural lands in Washington, Sylvester (60) found contributions of total phosphorus of 0.9-3.9 lb P per acre per year from surface drains and 2.5-8.9 lb P per acre per year from subsurface drains. Engelbrecht and Morgan (61) estimated the ortho- plus hydrolyzable phosphates from agricultural drainage in the Kaskaskia River basin, Illinois to range from 0-14.2 lb P per acre per year, with a mean of 0.35 lb P per acre per year. The estimates were made by measuring the amounts of phosphorus in the stream and subtracting the contributions from domestic and industrial wastes. Johnston et al. (62) reported 0.02-0.48 lb P per acre per year from subsurface drainage waters in a study for the San Joaquin Valley of California. Many factors, such as the type of soil and soil texture, topography, nature of crop or vegetation, nature and amounts of nutrients in and applied to the soil, intensity and distribution of rainfall, rates of infiltration and percolation, management practices, and probably some others, influence the transport of nutrients to surface waters by runoff and drainage from agricultural land as well as from uncultivated land. The contributions and amounts of nutrients from these sources would, therefore, be expected to be quite variable.

One of the growing problems of waste management and disposal is that concerned with farm animal wastes. With the trend towards raising animals in great numbers in small areas, such as feedlots and poultry farms, the problem is predicted to intensify. Wastes from these areas can be a significant source of nutrients entering surface waters.

Storm drainage from urban areas has recently become an important source of pollutants to receiving surface waters. Numerous investigations on the quality of storm waters have been made but only a few have included their content of nutrients. Weibel et al. (63) made an extensive study of the characteristics of stormwater runoff from a 27-acre residential and light commercial drainage basin with separate sewers in Cincinnati, Ohio and reported that the phosphates in the storm water amounted to 0.78 lb P per acre per year. This amount was estimated to be 9 per cent of the phosphorus in the raw domestic sewage from the area. A study by Sylvester (64) in 1959 and 1960 indicated that storm water in Seattle street gutters contained up to 0.78 mg P/l soluble phosphorus and up to 1.4 mg P/l total phosphorus.

The nutrient contribution to be expected from domestic and wild waterfowls has been estimated in a few investigations. Sanderson (65) reported a phosphorus contribution by domestic ducks of 0.4 lb of soluble phosphorus (as P) per duck per year and 0.9 lb of total phosphorus per duck per year. Paloumpis and Starrett (66) applied a factor of 0.5 to these figures and estimated the phosphorus contribution from the wild duck population to 3,500-acre Lake Chautauqua in Illinois to be 2.6 lb P per acre of water surface per year of soluble phosphorus and 5.6 lb P per acre per year of total phosphorus. From Gates' (67) studies of duck farm wastes, a phosphorus contribution of total phosphorus from 1.4-2.0 lb P per duck per year can be estimated assuming an average water usage per duck of 40 gallons per day.

In general, rainfall and dustfall can be expected to contain only small amounts of phosphorus. The phosphorus in the rain and dustfall that fall on land areas may be a part of the phosphorus contributed by runoff. The phosphorus in the rain and dustfall that fall on surface waters, however, would be a direct contribution and should be considered in any evaluation of phosphorus input. Some figures have been reported for the phosphorus content and contribution of rain and dustfall.

Tamm (68) reported that pure rainwater samples collected in an open area in the experimental forest of the Forest Research Institute, Bogesund, Sweden in October and November contained 0.03 mg P/l. Voigt (69) reported that rainwater samples collected in an open area in southern Connecticut from two storms contained 0.01 mg P/l. Weibel et al. (70) analyzed rainfall collected in Cincinnati, Ohio, an urban area, and in Coshocton, Ohio, a rural area. They found a total hydrolyzable phosphorus concentration of 0.08 mg P/l at Cincinnati but only 0.0003 mg P/l at Coshocton.

Johnson et al. (71) studied dustfall as a source of water quality impairment by exposing jars partially filled with distilled water for 3 months on the roofs of various residences and business establishments throughout the city of Seattle and its surrounding area. Water soluble phosphorus found during the 3-month study ranged from 0-0.45 lb per acre per year.

The concentration of phosphorus in waste waters and effluents is of primary importance in waste treatment for phosphorus removal. From the above discussion, it is obvious that the phosphorus concentration of domestic sewage is now 2-4 times its concentration before the advent of synthetic detergents, and this is supported by reported data. Rudolfs (55) in 1947 reported phosphorus concentrations of 12 domestic sewages ranging from 1.7 to 4.0 mg P/l with an average of 2.3 mg P/l. On the basis of a daily per capita sewage flow of 100 gallons, he calculated the daily per capita phosphorus contribution to vary from 0.65 to 1.48 grams as P. From his data and other data collected in the United States and abroad, Rudolfs concluded that the amount of phosphorus present in domestic sewage averaged between 0.87 and 1.3 grams per capita daily.

Owen (72) found 1.5 to 3.7 grams, with a median of 2.3 grams, of phosphorus contribution per capita per day in 1950 and 1951 in the raw sewages of nine Minnesota communities with populations varying from 1,200 to 940,000. The variation in the per capita contribution of the different communities was attributed to the several types and volumes of industrial wastes in the different sewages and the higher values observed over those reported by Rudolfs were attributed to the influence of the increased usage of phosphorus-containing detergents.

In 1956, Engelbrecht and Morgan (57) reported mean phosphorus concentrations varying from 5.4 to 11.5 mg P/1 and average per capita contributions from 2.0 to 5.3 grams of phosphorus per day in the effluents of three trickling filter plants and one activated sludge plant in Illinois. Fitzgerald and Rohlich (73) reported phosphorus concentrations ranging from 1.0 to 13.0 mg P/1 in the sewage of several U.S. and European communities. Orthophosphate levels in the sewage plant effluents of seven California communities ranging from 1.2 to 21.2 mg P/1, with an average of 8.5 mg/1, were reported by Stone and Merrell (74) in 1958.

Nesbitt (75) reviewed published data and concluded that the orthophosphate concentration of municipal sewage effluents after complete treatment averages approximately 8.0 mg P/1. In a study in 1964-65 of the sources of phosphorus in the sewage of San Ramon, a small residential community in California, Jenkins (59) estimated the per capita contribution to be 3.68 grams of phosphorus per day of which sixty per cent was determined to originate from synthetic detergents. Jenkins and Menar (76) found mean total phosphorus concentrations of 10.2 and 13.8 mg P/1 in the raw sewages of two California sewage treatment plants, respectively, based on composite samples over a five-day period in 1966.

Buzzell and Sawyer (15) found average total phosphorus in the raw sewages of five Massachusetts cities and one city in New York ranging from 3.7 to 13.0 mg P/1 in 1967. Mean monthly total phosphorus levels ranging from 8.8 to 16.6 mg P/1 in the raw sewages of nine sewage treatment plants in Texas for 1964-65 were reported by Vacker *et al*. (77).

Some of the reported phosphorus concentrations above are summarized in Table IV.

PHOSPHORUS IN ALGAL METABOLISM

Microbiological nutrients are generally classified into two categories: macronutrients, which are required in relatively large quantities, and micronutrients, which are needed in only trace amounts. Phosphorus is one of the essential macronutrients and is a universal constituent of cell protoplasm. Phosphorus compounds are essential to many cellular functions and are an important part of several cell components such as cell membranes, coenzymes, nucleic acids, and nucleoproteins. Katchman (78) has listed the following as the most important functions of phosphorus compounds in the biochemistry of living cells:

1. In the basic processes of photosynthesis and metabolism, the synthesis and degradation of saccharides and other compounds proceed via phosphated intermediates.

2. Certain condensed phosphates and related compounds,

TABLE IV

PHOSPHORUS CONCENTRATION OF DOMESTIC SEWAGE

Phosphorus Conc., mg P/1		Year Reported	Reference
Raw Sewage	Treatment Plant Effluent		
1.7-4.0		1947	Rudolfs (55)
	5.4-11.5	1956	Engelbrecht & Morgan (57)
1.0-13.0		1958	Fitzgerald & Rohlich (73)
	1.2-21.2	1958	Stone & Merrell (74)
	8.0	1966	Nesbitt (75)
10.2, 13.8		1967	Jenkins & Menar (76)
3.7-13.0		1967	Buzzell & Sawyer (15)
8.8-16.6		1967	Vacker *et al.* (77)

* Saw—

particularly the ubiquitous adenosine triphosphate (ATP), act as strong phosphating agents thereby converting potentially endergonic (energy-requiring) reactions into exergonic (energy-yielding) ones.

3. The pyrophosphate moiety is found in the structure of a number of important coenzymes although the chemical action of these substances does not involve the phosphate part.

4. The nucleic acids involved in cell reproduction and synthesis of proteins exhibit a spiralled-rod structure with the backbone of the spiral being made up of alternating orthophosphate and sugar radicals.

One of the most important functions of phosphorus compounds in the metabolism of algae and all living cells is the dominant role that they occupy in energy transformations. The two principal agents of energy transfer within the cell both involve phosphorus-containing compounds. These agents are the adenylic acid system (AMP, ADP, and ATP, collectively) and the pyridine nucleotides (DPN and TPN). The adenylic acid system serves as the carrier of biochemical energy associated with energy-rich phosphate bonds. The pyridine nucleotides are co-enzymes which are involved in metabolic oxidation-reduction reactions (electron transport).

In energy transformations within the cell, adenosine triphosphate (ATP) is the supplier of energy to endergonic biochemical reactions and is also the 'storehouse' of the energy obtained and transformed by the cell from various sources. The energy is stored in ATP in its terminal pyrophosphate linkage (P-O-P).

The synthesis of the pyrophosphate bond of ATP is an essential stage in the energy transformations of living cells. ATP is formed when adenosine diphosphate (ADP) accepts a high-energy phosphate group from one of several metabolic 'donors' and binds it in the terminal pyrophosphate linkage of ATP. It is well established that only ATP serves as the energy supplier, by transferring its terminal phosphate group to a suitable phosphate acceptor, in most phosphorylation reactions requiring an input of energy.

The pyridine nucleotides, diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN), are coenzymes which function in association with enzymes known as dehydrogenases, as carriers of hydrogen or electrons in the enzymatically-catalyzed oxidations and reductions of biological substrates. DPN and TPN are also known, respectively, as coenzyme I and coenzyme II and, in recent biochemical literature, as nicotinamide-adenine dinucleotide (NAD) and nicotinamide-adenine dinucleotide phosphate (NADP). DPN has the structure of adenosine (adenine-ribose) and nicotinamide ribose linked by a pyrophosphate group. TPN has the same structure as DPN except that the adenosine moiety of the DPN molecule carries an additional phosphate group in the 2'-position.

DPN and TPN serve as hydrogen or electron carriers in oxidation-reduction reactions by undergoing reversible oxidation and reduction of the nicotinamide part of the molecule. This role in hydrogen transport is the biological function of nicotinamide, a well-known member of the vitamin B group. The general mode of action of DPN or TPN in oxidation-reduction reactions can be represented as follows:

$$AH_2 + \text{DPN (or TPN)} \rightleftharpoons A + \text{DPNH}_2 \text{ (or TPNH}_2\text{)}$$

in which AH_2 and A are, respectively, the reduced and oxidized forms of the metabolic substrate and $DPNH_2$ and $TPNH_2$ are, respectively, the reduced forms of DPN and TPN. In the presence of a suitable dehydrogenase enzyme D_1, the reduced form of the substrate, AH_2, transfers two hydrogen atoms (electrons) to DPN or TPN. The $DPNH_2$ or $TPNH_2$ formed in the reaction now becomes linked with another dehydrogenase enzyme D_2, which catalyzes the transfer of two hydrogens from the reduced coenzyme to an oxidized substrate, B. Reactions of this type are among the most important reactions in living cells and have long been known to play a key role in glycolysis, fermentation, respiration, and, recently, also in photosynthesis.

The phosphate groups of DPN or TPN are not involved in the oxidation and reduction of the coenzyme. Nevertheless, it is well established that certain dehydrogenase enzymes are specific for DPN while others are specific for TPN. Since the third phosphate group is the only difference between DPN and TPN, it appears that this phosphate group plays a role in the attachment of the coenzymes to the substrate or to the enzyme.

Phosphorus compounds are intimately involved in photosynthesis and algal metabolism. Excellent accounts of the conceptual and experimental development of phosphorus metabolism in photosynthesis have been given by Arnon (79, 80) and Kandler (81).

Photosynthesis may be defined as the conversion of radiant energy to the chemical energy required for cell growth and metabolism. From the standpoint of metabolism, it may be visualized as the partial reduction of carbon to provide the requisite cellular metabolites for growth (78). The photosynthetic process may be represented as follows:

$$6CO_2 + 12H_2O^* \xrightarrow{h\nu} C_6H_{12}O_6 + 6O_2^* + 6H_2O$$

Photosynthesis is generally agreed to be an endothermic reaction involving (a) the absorption of radiant energy (visible light) by the living cell, (b) utilization of the energy absorbed for the photolysis of water and the concomitant production of a photoreductant, and (c) assimilation of CO_2 and its conversion to carbohydrate, with simultaneous production of molecular oxygen.

Phosphorylation steps with ATP are essential in the series of reactions leading to the formation of carbohydrates, the main products of the overall photosynthetic process (80). As in respiration, the adenylic acid system is the most important storage form of biochemical energy. Energy-rich phosphorus compounds are formed during the photochemical reactions of photosynthesis and are used in the dark reactions of CO_2 reduction. The energy captured and transformed from light in the photochemical reactions is stored mainly in ATP and is used in this form to support all energy-requiring metabolic reactions, such as CO_2 assimilation, activation of amino acids in protein synthesis, formation of nucleic acids, and synthesis of inorganic polyphosphates (80). The pyridine nucleotides are the reducing agents and phosphorylated compounds are intermediates in carbon dioxide assimilation. Inorganic polyphosphates may make up a significant part of the phosphorus in algal cells, and these compounds appear to also have important metabolic roles.

Condensed inorganic phosphates, particularly the linear inorganic polyphosphates (polyP), is one form in which phosphorus compounds may be present in microorganisms. The occurrence of polyP in a variety of microorganisms and their accumulation under nutritional conditions unfavorable to growth have been described by numerous investigators (82). Arnon (79) stated that these compounds appear to be normal constituents of green cells. Kuhl (83) has stated that polyP comprise a large part of the phosphorus compounds in algae. According to Harold (82), considerable quantities of polyP occur in Chlorella and Euglena under normal growth conditions.

The most recent literature on the metabolism of inorganic polyphosphates have recently been reviewed by Harold (82). The most striking aspect of polyP metabolism is the variability of the polyP content of cells. In phosphate-starved cells, polyP cannot usually be detected; at the other extreme, it may accumulate to the point where it is the most abundant cellular phosphorus compound. In algae, as well as in many other microorganisms, the polyP content is a function of the growth phase; it is lowest in the exponential phase, highest in older cultures, and a reciprocal relationship between the RNA and polyP contents appears to exist (82, 84).

Various metabolic roles and functions have been proposed for the inorganic polyphosphates. Following the demonstration by Yoshida and Yamataka (85) and by Kornberg et al. (86, 87) of an enzyme which catalyzes the reversible transfer of phosphate between polyP and the terminal group of ATP, it was proposed (87, 88, 89) that polyP serves as a microbial phosphagen, analogous to creatine phosphate in mammalian muscle, or an energy store. PolyP accumulate when ATP production exceeds the cellular energy demand for work and biosynthesis according to the following equation:

$$ATP + (PO_3^-)_n \rightleftharpoons ADP + (PO_3^-)_{n+1}$$

Some support for this theory has been provided by the works of Meyerhof et al. (90) and Yoshida (91), which showed that the P-O-P bonds of the polyphosphate molecule are so-called energy-rich phosphate bonds.

Polyphosphates have also been shown to serve as a source of phosphorus for the biosynthesis of nucleic acids and phospholipids under conditions of phosphorus starvation (92, 93). The polyP that accumulate under appropriate conditions may suffice for several doublings of the cell mass during subsequent phosphorus starvation. Possibly of greater significance, polyP could constitute an accessible reserve for the synthesis of messenger RNA, ribosomes, and metabolic intermediates to facilitate the initiation of growth.

Another postulate of the physiological role of polyP is that it is involved in the regulation of the inorganic phosphate, ATP, and ADP levels in cells (82). An ill-defined relationship between polyP and cell division, the polyP serving either as an energy pool or as a regulator of the cation content, has also been proposed by several investigators (94, 95, 96, 97).

PHOSPHORUS REQUIREMENTS AND CONTENT OF ALGAE

In the use of algae as an agent for removing nutrients from solution, one must consider their requirements for and the cellular content of the nutrients of concern as these factors determine in large measure the assimilation capacity of a system and the amounts of materials that can be removed. The mineral requirements of algae have been the subject of numerous studies and several reviews dealing wholly or partly on the subject have appeared (98-102).

Ketchum (99) defined four ways in which nutrient requirements have been studied and expressed: (a) the absolute, (b) the normal, (c) the minimum requirements, and (d) their optimum characteristics. The absolute requirement for a nutrient is based on the postulate that the algae cannot grow, reproduce, or photosynthesize if the nutrient is absent in the environment and no nutrient can be substituted for it. The normal requirement is the amount of each nutrient contained in cells produced during active growth of a population while no nutrient is limiting. This requirement is based on the postulate that there is an ideal or normal composition of the cell, and the algae tend to produce such cells if the environment permits. Ketchum stated that the normal requirement is as yet, and may always be, ill-defined because of the variability of the composition of phytoplankton. Furthermore, the cells may, by selective absorption, assimilate a large proportion of an element in limited supply without assimilating more than a small proportion of a nutrient present in excess. As a result, variations in the composition of the cells tend to be smaller than variations in the composition of the medium.

The minimum requirement is the quantity of a nutrient in the cell when the nutrient is limiting to the growth of the population, all other nutrients being present in excess. It determines the mass or number of cells that can be produced from a given quantity of nutrient, provided light or some other environmental factor does not limit the growth of the population. Cells containing the minimum quantity of a nutrient are deficient cells which could contain a larger amount if the nutrient were available.

The optimum concentration or range of concentrations is that which will permit the maximum rate of growth, reproduction, or photosynthesis of an algal population. It is not certain whether the same concentration will be optimal for these three criteria. The optimum concentration is based on the concept that too low a concentration may be limiting because of decreased rate of assimilation, and too high a concentration may be inhibitory or toxic.

Ketchum and Redfield (103) reported that the normal requirement for phosphorus by various Chlorophyceae was 2 to 3 per cent of the dry weight of cells. The phosphorus content of cells can, however, vary considerably since cells higher or lower in phosphorus than normal can be produced by changing the composition of the medium in which they are grown. Phosphorus deficiency in various algae have been reported by Franzew (104), Ketchum (105), Scott (106), Mackereth (107), and Al Kholy (108). Luxury consumption with storage of excess phosphorus has been observed by a number of investigators (109, 110, 111).

Cells deficient in phosphorus can be produced by allowing cultures to continue growing and reproducing in the light after all the phosphate in the culture medium has been assimilated. Franzew (104) reported producing in this way deficient cells of Scenedesmus quadricauda and Pandorina morum which contained only as little as one-fifth of the normal phosphorus content. In a normal nutrient solution, Scenedesmus quadricauda contained 8.7×10^{-6} µg P per cell, after three days in a phosphate-free solution the phosphorus content dropped to 0.92×10^{-6} μg P per cell. Ketchum (105) studied the development and restoration of phosphorus deficiency in Nitzchia closterium and Chlorella pyrenoidosa and showed that the phosphate deficient cells contained only about one-fifth of the phosphorus found in normal cells. When the deficient cells were again placed in a medium containing phosphate, rapid phosphorus uptake resulted and the phosphate debt incurred was restored. The phosphate-deficient cells could absorb phosphate both in the light and in the dark whereas normal cells did not absorb phosphate in the dark. The phosphate absorbed by the deficient cells was directly proportional to the number of cells present and independent of the phosphate concentration in the medium.

Scott (106) investigated the behavior of phosphate-deficient Chlorella pyrenoidosa cells during a restoration period when adequate phosphate was supplied to the nutrient medium. He found that the phosphate absorbed by the deficient cells either in the light or in the dark was independent of the concentration of phosphorus in the medium except at the limiting concentrations of phosphate normally required by the cell. At the levels limiting for growth, the phosphate uptake was proportional to the phosphate concentration. Similar findings were reported by Mackereth for Asterionella formosa (107).

Rohde (111) reported that in addition to satisfying their phosphate debt by assimilation in the dark, Scenedesmus quadricauda could also store excess phosphorus when adequate supplies are available. Such cells, when returned to light in a phosphate-free medium, were able to grow as rapidly as those in an adequate medium. The phosphorus uptake to satisfy the deficiency proceeded very rapidly (one day), but accumulation of extra phosphorus required a fairly long time (7 days).

Al Kholy (108) measured the assimilation of phosphorus and the growth of phosphate-deficient cells of Chlorella pyrenoidosa after the addition of phosphate to the deficient culture and found that growth continued until the phosphorus content of the cells dropped to a minimum value of 0.10×10^{-6} µg P per cell. A maximum value of 1.5×10^{-6} µg P per cell was found for the phosphorus content of the cells.

Ketchum (112) studied the absorption of nitrate and phosphate by illuminated cultures of Nitzchia closterium and showed that the rate of phosphorus assimilation was directly related to the phosphorus concentration below 0.1 mg P per liter and also to the concentration of nitrate nitrogen below 0.2 mg N per liter. In these experiments, phosphorus concentrations less than 0.017 mg P/1 limited cell division as well as the rate of assimilation. Variable amounts of phosphorus contained per cell were also found by Lund (109) in Asterionella formosa. The difference was attributed by him to the storage of excess phosphorus by cells grown in phosphate-rich media.

Using radioactive phosphorus, Goldberg et al. (110) found the relationship between the phosphorus content of the diatom Asterionella japonica during exponential growth and that of the medium to be linear within the range of 0.015 to 0.110 mg P/1. The range of variation within the cell, however, was only about one-half of the range in the medium.

Using the isotope method, Knauss and Porter (113) determined the quantities of various elements present in Chlorella at varying concentrations of the elements in the medium. The elements studied were P, Ca, S, Fe, Mn, Zn, Cu, and Sr. They found that the absorption by the algae of all elements, except P and S, was directly proportional to the concentration of the element. The quantities of P and S in the algal cells were proportional to the concentration of the element in the medium only when the concentration was below 200 mg/1 for P and 2 g/1 for S, and were constant at the higher concentrations of the elements. Their data showed that the phosphorus in the algae was 1.35 to 2.34 per cent of the dry weight of the cells when the phosphorus concentration in the medium was 27.5 to 200 mg/1. Concentrations of the elements within cells at growth-limiting levels of nutrient concentration were not determined.

The physiological state of algal cells may also influence their phosphorus content. Ketchum and Redfield (103) reported that old dense cultures of Stichococcus bacillaris had higher cellular phosphorus than young cultures, however, Chlorella pyrenoidosa did not show any progressive change in phosphorus content with age of the culture.

The minimum phosphorus content or requirement has been reported for some algae. Lund (109) found the minimum requirement of Asterionella formosa to range from 6×10^{-8} to 4×10^{-6} μg P per cell. Mackereth (107) reported a minimum value of 6×10^{-6} μg P per cell which closely corresponds to the value found by Lund. For Asterionella japonica, Goldberg et al. (110) found a minimum amount of 5×10^{-8} μg P per cell, which is also in the same range. A minimum requirement of 1×10^{-7} μg P per cell was determined by

Al Kholy (108) for Chlorella pyrenoidosa.

Using pure chemicals, Chu (114) investigated the requirements of various planktonic algae and found that the requirements for N and P agreed well among the different species that he used, although there were minor differences in the lower and upper limits that were suitable. All the algae that he studied fluorished in media containing 1 to 7 mg N/1 and 0.1 to 2 mg P/1. He stated that the algae are likely to suffer from a deficiency when the concentration of N falls below 0.2 mg/1 and that of P below 0.05 mg/1, and from an inhibiting effect when the concentration of N and P exceeded 20 mg/1. The optimum range of phosphorus concentration was found to be wider when nitrate was used than when ammonia was used as the source of nitrogen. In another investigation, Chu (115) found that the optimum growth of all the organisms could be obtained in phosphorus concentrations from 0.09 to 1.8 mg/1, while a limiting effect on all of them occurred in phosphorus concentrations from 0.009 mg/1 downwards, and marked inhibiting effect in phosphorus concentrations from 45 mg/1 upwards. The lower limit of the optimum range of phosphorus concentration varied from about 0.018 mg/1 (Nitzchia palea, Tabellaria flocculosa) to about 0.09 mg/1 (Pediastrum boryanum, Staurastrum paradoxum, Botrycoccus braunii). The upper limit varied from 8.9 mg/1 (Nitzchia palea, Tabellaria flocculosa) to 17.8 mg/1 (Pediastrum boryanum, Staurastrum paradoxum, Botrycoccus braunii) when nitrate was the source of nitrogen, while it lay at about 1.78 mg/1 for all the planktonts when ammonia was the nitrogen source. The upper limits of nitrogen and phosphorus concentrations for optimum growth of the plankton organisms studied were always higher than the highest concentrations found in ordinary waters and Chu concluded that the growth of the organisms is unlikely ever to be unfavorably affected by too high a concentration of nitrogen and phosphorus.

In a study of the mineral nutrition of *Coccochloris peniocystis*, Gerloff *et al*. (116) found the minimum concentration of phosphorus for optimum growth to be 0.45 mg/l. The highest concentration tested by them was 5.4 mg P/l and was found to be not inhibitory.

Rohde (111) found rather wide variation in the phosphorus requirements of different plankton forms. He noted that the "content of P varies within the interval below 20 µg P/l." From this and other field observations and laboratory data on oligotrophic organisms, he divided phytoplanktonts into three categories with respect to phosphorus requirements: (a) low requirement: upper limit less than 20 µg/l, (b) medium requirement: lower limit less than and upper limit greater than 20 µg P/l, and (c) high requirement: lower limit greater than 20 µg P/l. However, the concentrations of N and P which produced optimal growth *in vitro* were generally between 10 and 100 times as much as those which produced similar growth in nature.

Rohde (111) found that the addition of 5 µg P/l to Lake Erken water was sufficient to inhibit the growth of two Chrysophyceae, *Dinobryon* and *Uroglena;* addition of only 2 µg P/l gave maximum growth of *Asterionella formosa* but addition of 50 or more µg P/l inhibited growth; addition of 500 to 1000 µg P/l was necessary to obtain maximum growth of *Scenedesmus*.

Kuenzler and Ketchum (102) reported that the cellular phosphorus content of *Phaeodactylum tricornutum* may vary from as high as 6.6×10^{-14} mole per cell to as little as 0.2×10^{-14} mole per cell when phosphorus is scarce enough to limit growth. The organism was found to have the ability to absorb phosphate until the concentration in the medium is as low as 7×10^{-15} M.

Some typical values of the phosphorus content reported for various algae are given in Table V.

MECHANISMS OF ION UPTAKE

The uptake of materials from their environment is one of the most characteristic and important processes of living organisms. The kinds and amounts of mineral elements absorbed are affected by various factors, including the kinds and concentrations of elements in the environment, and also vary among species. The process is complicated and the mechanisms appear to be as yet not completely understood. General reviews and discussions of mineral uptake by plant cells have been given by Kramer (118) and Robertson (119).

An early view which persisted for some time regarding the absorption of nutrients by plants was that the various substances found in the cells entered by diffusion. However, this belief was abandoned when it was found that some substances occurred in cells in higher concentrations and others in lower concentration than they occur in the environment. Current concepts view the entry of ions to be not dependent merely on their diffusion but is the result not only of the complicated ionic matrix which constitutes the cytoplasm at any time, but also of processes dependent on the cellular metabolism (119). Any diffusion which occurs through the cell walls is regarded as an incidental or preliminary process rather than

TABLE V

PHOSPHORUS CONTENT OF VARIOUS ALGAE (117, 2)

Material Analyzed	Percent of Dry Weight
Microcystis sp.*	0.52
Anaebaena sp.*	0.53
Microcystis aeroginosa+	0.69
Aphanizomenon sp.#	0.6
Oscillatoria brevis	1.28
Anabaena sp.	1.20
Lyngbya hemensii	1.07
Chlorella vulgaris**	1.1
Chlorella pyrenoidosa++	0.94-1.5
Scenedesmus bijugatis	0.53-1.8
Stigeoclonium stagnatile##	1.89
Calotrix sp.	1.18
Euglenoid algae***	1.4
Scenedesmus quadricauda***	2.2

*Birge and Juday (1922)
+Gerloff and Skoog (1954)
#Phinney and Peek (1961)
**Geoghegan (1953)
++Scott (1943)
##Bogan et al. (1960)
***Oswald et al. (1964)

directly related to the accumulation of ions.

Many investigators have regarded the accumulation of ions as occurring in two stages: (a) a physical or non-metabolic process involving exchange adsorption at the cell surface, and (b) a metabolic step in which active transport of ions occurs across the differentially permeable membrane of the cell. The first step depends on the laws of Donnan equilibrium; that is, the ionic equilibrium established between two phases, one of which contains immobile ions. Cell walls and cytoplasm both contain immobile anions and establish Donnan equibria with the external solution. Adjustments between mobile ions of cells and the surrounding medium are known as ion exchange. Ions held by polar linkages to charged groups on solid structures of cells or medium are held by adsorption and are available for exchange. Active transport moves ions into cells or through tissues against a concentration gradient.

Another mechanism by which ions may be removed from solution is by combination or entering into the formation of organic molecules. The macronutrient anions, nitrate, phosphate, and sulfate, appear to enter cells not by exchange but by combination and active transport.

Robertson (119) has remarked that it is impossible to generalize about the absorption mechanism; absorption of an ion which combines with organic molecules like phosphate may be a distinct mechanism from that of an ion like bromide. The state of the mineral nutrient determines the mechanism by which they enter the cell. The following have been put forth as principal steps in absorption of ions by plant cells (119):

1. Mobile cations enter largely to balance immobile anions in cells; other mobile cations must leave simultaneously.

2. Where the concentration of immobile anions is high, the concentration of cations is also high.

3. Mobile anions enter, but not as readily as mobile cations, because of the immobile anion concentration. Mobile anions enter where required to combine in a metabolic process, e.g., phosphate and nitrate. Such anions are also in relatively high concentrations in actively metabolizing, i.e., growing regions.

4. Both mobile cations and anions are accumulated by the active transport mechanism which is associated particularly with the vacuolated cells.

FACTORS INFLUENCING PHOSPHORUS UPTAKE

The absorption of phosphorus or other nutrients by algae from the surrounding medium is influenced by several factors. Because phosphorus absorption is intimately involved in algal photosynthesis, growth, and metabolism, factors affecting these activities should also influence phosphorus uptake in some way. However, phosphorus uptake may not necessarily be growth-related. Important external factors affecting algal phosphorus uptake are light, carbon dioxide, pH, temperature, composition of the medium, form and concentration of phosphorus, presence and concentration of other ions and organic compounds. Intrinsic factors such as algal genera and species, age of the culture, physiological conditions of the algae, etc. may also affect phosphorus uptake. The effects of some of these factors have been indicated earlier.

Many studies on the influent of light and CO_2 on phosphorus absorption have been made in studies concerned with phosphorus metabolism in photosynthesis. Using P^{32}, Gest and Kamen (120) found both a considerably greater uptake and turnover of inorganic phosphate in the light than in the dark in Chlorella pyrenoidosa, Scenedesmus (D_3 strain), and Rhodospirillum rubrum. Wassink et al. (121) found that in the light purple sulfur bacteria (Chromatium) and Chlorella vulgaris cells absorbed more inorganic phosphate from the external medium in the presence of CO_2 than in its absence. Similar results were reported by Nihei (122) for Chlorella pyrenoidosa. On the other hand, Emerson et al. (123) found that phosphorus uptake by Chlorella pyrenoidosa in the light was promoted only in the absence of CO_2. Greater absorption of phosphate in the light than in the dark by phosphate-deficient cells of Nitzchia closterium and Chlorella pyrenoidosa were reported by Ketchum (105) and Scott (106).

The pH of the culture medium can influence the rate of phosphorus uptake through effects on growth and availability of the nutrient. In addition, the pH determines the ionic species of the nutrient and may also affect the cell characteristics and mechanisms for phosphate absorption. Scott (124) determined the mineral composition of Chlorella pyrenoidosa at various pH values and reported that the cells contained more phosphorus at the higher pH. Wintermans (125), however, found that, in the absence of CO_2, illuminated cells of Chlorella vulgaris fixed phosphorus only in acid media (around pH 4). In further studies on this organism, Badour (126) showed that the influence of external pH on the rate of uptake of phosphorus and on its subsequent distribution into various fractions was different in light and in the dark. Mackereth (107) reported that the uptake of phosphate by Asterionella formosa was greatest at pH values between 6 and 7.

The temperature can also affect phosphorus uptake through its influences on the rates of algal growth and metabolism. The effects of temperature on photosynthesis and algal growth rate are complicated and depend on other factors such as carbon dioxide, light, etc. In general, an increase in temperature up to some maximum results in increased growth rate and metabolism, and above such a maximum there is a rapid decline (127).

As mentioned earlier, the concentration of phosphorus in the medium has been found by several investigators to have some influence on the rate of phosphorus uptake by algae. Ketchum (112) found the rate of phosphorus assimilation by Nitzchia closterium to be directly related to the phosphorus concentration below 0.1 mg P/1. Phosphorus concentrations less than 0.17 mg P/1 limited cell diversion as well as the rate of assimilation. Scott (106) reported that phosphate absorption of phosphate-deficient cells of Chlorella pyrenoidosa in the light or in the dark was dependent on the concentration of phosphorus in the medium at the limiting concentrations normally required by the cells. Gest and Kamen (120) showed the phosphorus content of Chlorella pyrenoidosa and Scenedesmus (D_3 strain) cells to depend strictly on the concentration of phosphate in the medium. Chu (115) found lower and upper limits of phosphorus for optimum growth of various planktonic algae. Knauss and Porter (113) found that the phosphate absorbed by Chlorella pyrenoidosa was proportional to the concentration of phosphorus in the medium when this was below 200 mg/1.

Working with diatoms, Goldberg (110) also found the phosphate uptake to be proportional to the concentration in the medium over the range 0.5 to 3.5 µg/1.

The composition of the medium and the concentration of other ions are other factors which may also affect phosphorus uptake. Chu (115) found that the upper limit of phosphorus concentration for optimum growth of several fresh-water algae varied according to the composition of the medium. Also, the upper limit for optimum growth was higher when nitrate was supplied than when ammonium salt was supplied as the nitrogen source. Scott (106) reported that Chlorella pyrenoidosa absorbed potassium and phosphorus in a ratio of one atom of potassium to one atom of phosphorus, suggesting that these elements combine chemically with some constituent in the cell. Ketchum (112) noted that the rate of phosphorus uptake of Nitzchia closterium was related not only to the phosphorus concentration when this was below 0.1 mg P/1 but also to the concentration of nitrate when it was below 0.2 mg N/1. In experiments with Scenedesmus obliquus, Krauss and Thomas (128) noted that during the early stages of growth in a medium deficient in micronutrients, the phosphorus content of the cells was considerably higher than that of normal cells. The individual micronutrient responsible for this effect was, however, not determined. Rohde (111) found that the concentrations of nitrogen and phosphorus needed to produce optimum growths in vitro were generally 10 to 100 times as much as those which produced similar growth in natural waters and concluded that there must be some growth factors in natural waters that are lacking in synthetic media.

PHOSPHORUS REMOVAL EFFICIENCIES OF CONVENTIONAL WASTE TREATMENT

The capacity or efficiency of so-called conventional (primary and complete) waste treatment for removing phosphorus has become of interest only recently, with the recognition of the significance of domestic sewage as a major source of fertilizing materials to surface waters and the need for controlling the quantities of nutrients from this source. As a result, relatively few studies have been made to determine the phosphorus removal efficiency of conventional treatment. Rudolfs (55) reported removals of 50-60 per cent by settling, 75-80 per cent by filtration, and 80-90 per cent by the activated sludge process in 1947, based on samples from a dozen different domestic sewages and results from England, Germany, and Russia. These are rather high efficiencies, however, it should be appreciated that they were based on raw sewage phosphorus concentrations that are very much lower than present-day levels.

Owen (72) found phosphorus removals varying from 2 to 46 per cent, with an average of 23 per cent and a median value of 20 per cent, in nine Minnesota treatment plants in 1950-51. Both primary and secondary plants were included in the study. The higher removals were obtained generally in the plants with secondary treatment, which accomplished the highest degrees of BOD reduction.

Ohle (129) reported that sewage treatment by sedimentation and percolating filters had little effect on the total phosphorus concentration.

Stones (130, 131, 132) has also reported on phosphorus removal efficiencies of sedimentation, biological filtration, and activated sludge treatment. Settling for 12 hrs of a sewage with an average phosphorus concentration of 5.73 mg P/1 gave an average removal of 23 per cent. Average removals of 30 and 32 per cent, respectively, were obtained with 18 hrs settling of sewages containing average phosphorus concentrations of 6.25 and 6.67 mg P/1. Biological filtration without settling of the filter effluent removed only 14 per cent of the phosphorus in the influent to the filter (4.36 mg P/1), settling of the filter effluent for one hr increased the removal to 31.6 per cent of the filter influent phosphorus. Activated sludge treatment produced average removals of 64-71 per cent of the settled sewage fed to the aerators. The settled sewage had an average concentration of 5.14 mg P/1.

In a study at several full-scale sewage treatment plants in California of the efficiency of primary sedimentation for removing phosphorus, Jenkins and Menar (76) found that the primary sludge contained only 0.6 to 0.7 per cent phosphorus, dry weight basis. The per cent removal of phosphorus was found to increase slightly with increased efficiency of suspended solids removal. They concluded that for sewages with an average phosphorus concentration of about 10 mg P/1, only 5 to 15 per cent (with an average of 10 per cent) of the phosphorus can be removed by primary sedimentation and the higher removals can only be obtained in efficiently operated (50 to 60 per cent suspended solids removal) sedimentation basins.

Phosphorus removal by activated sludge treatment were also studied by Jenkins and Menar (76). Using an activated sludge pilot plant at the Engineering Field Station of the University of California at Richmond, Calif., they determined the performance of the activated sludge process in removing phosphorus at a wide range of organic loading rates. A significant finding in the study is that the phosphorus content of the activated sludge solids did not vary significantly over a range of substrate removal rate of 0.3 to 42.5 lb COD removed/lb VSS/day, with a mean weighted value of 2.62 per cent of the volatile suspended solids. Based on this value and activated sludge process kinetic data derived in the study, they concluded that the maximum phosphorus removal biologically by activated sludge treatment from primary sewage with a phosphorus content of 9 mg P/l would not exceed 20 per cent at loadings normally employed in practice (0.1 to 0.3 lb COD removed/lb VSS/day).

Monthly average phosphorus removal efficiencies ranging from 2 to 55 per cent in 1964-65 were reported by Vacker *et al.* (77) for 8 activated sludge plants in Texas. Three-fifths of the reported values, however, were 22 per cent or less. Exceptionally high phosphorus reductions (close to 90 per cent) were observed at the San Antonio, Texas, Rilling, plant and these were ascribed by these workers to "luxury consumption" of phosphorus by activated sludge organisms.

PROCESSES FOR PHOSPHORUS REMOVAL FROM WASTE WATER

In this section a brief summary is made of reported work on phosphorus removal processes to give an indication of the "state of the art" of phosphorus removal. Methods that have received attention include chemical precipitation, ion exchange, modifications of the activated sludge process, combined chemical-biological treatment, the use of algal systems, electrochemical treatment, and effluent irrigation on land. General reviews of reported studies have appeared frequently in recent years (28, 31, 75, 133-137).

Chemical Precipitation

Removal of phosphorus from sewage and treated effluents by chemical precipitation has been studied with a number of chemicals including lime, aluminum sulfate, ferric sulfate, ferrous sulfate, ferric chloride, sodium aluminate, and activated silica. Rudolfs (138) studied coagulation with lime to remove phosphates from sewage and reported the process to be effective. Sawyer (56) reported that Rudolf's findings were confirmed in studies at the Sedgwick Memorial Laboratories in Massachusetts and that similar studies with sewages enriched with the complex phosphates, sodium tripolyphosphate and tetrasodium polyphosphate, showed these compounds to be also readily removed without any appreciable increase in the lime requirements. His results showed that the total soluble phosphates in the unenriched and enriched effluents were reduced to about 0.5 mg P/l with a dosage of 280 mg/l $Ca(OH_2)$.

From laboratory studies using lime on secondary effluent, Owen (72) concluded that phosphorus could be almost completely removed from treated effluent by a dosage of lime sufficient to raise the pH to approximately 11. A lime dosage of 600 mg/l CaO produced a pH of 10.95 and reduced the phosphorus of a secondary effluent from 6.0 to 0.25 mg P/l after one hr of settling or a 96 per cent reduction, and to 0.015 mg P/l, or a reduction of more than 99 per cent, after an 18-hr settling period. The laboratory results were substantially confirmed in plant scale tests carried out in a 1 mgd high rate-low rate trickling filter plant. A dosage of 720 mg/1 $Ca(OH)_2$ (545 mg/1 as CaO) added to the influent to the final settling tank reduced the total phosphorus in the influent from 7.4 to 1.7 mg P/1, or a reduction of 77 per cent, and a dosage of 360 mg/1 $Ca(OH)_2$ reduced the phosphorus by 67 per cent. In a test in which the high rate filter was bypassed and the phosphorus in the final tank influent was 8.3 mg P/1, a lime dosage of 720 mg/1 $Ca(OH)_2$ removed 86 per cent of the tank influent phosphorus. Owen remarked that the phosphorus removals would have more closely approached the results obtained in the laboratory with proper facilities for efficient mixing, flocculation, and settling.

Stones (132) reported chemical precipitation experiments on raw sewage employing 400 mg/1 of lime (CaO), aluminum sulfate, and sulfuric acid. Average removals of 88 and 94 per cent were obtained with lime and alum, respectively, of the 6.25 mg P/1 present in the raw sewage, but acid precipitation produced an average removal of only 27 per cent.

Malhotra et al. (139) reported a laboratory study on nutrient removal from secondary effluent by alum flocculation, lime precipitation, and by pH adjustment using NaOH in 1964. The experiments were conducted in accordance with conventional jar test procedures employing activated sludge effluent from the Madison, Wisconsin, Nine Springs Sewage Treatment Plant. They found all the chemicals to be effective in removing phosphorus but concluded that lime was the most suitable chemical for the particular effluent used on the basis of chemical cost. A dosage of 600 mg/1 $Ca(OH)_2$ was required ro raise the pH to 11 and this dosage removed 99 per cent of the total phosphorus. The percentage removal by lime was found to be independent of the initial phosphate concentration in the concentration range of 7 to 35 mg P/1.

In another laboratory study, Karanik and Nemerow (140) determined an optimum lime dosage of 300 mg/1 to remove algal nutrients from raw sewage. A reduction of greater than 93 per cent of the phosphates in the raw sewage was obtained with this dosage.

Lime treatment of raw sewage to remove nutrients was also investigated in the laboratory by Buzzell and Sawyer (15) and they reported that the process could remove 80-90 per cent of the total phosphorus with better than 97 per cent removal of soluble inorganic forms. The lime requirement was found to be independent of the phosphorus content and could be estimated from the alkalinity. Lime dosage could be controlled by measurement of the pH and they recommended determination of the optimum pH for each individual case. The volume of sludge produced was about one per cent of the volume of sewage treated. They stated that chemical conditioning of the sludge to improve its filterability may be necessary in some cases.

Alum has been found to be the most suitable phosphate precipitant by a number of investigators. Lea et al. (141) in 1954 determined aluminum sulfate to be the most suitable coagulant for removing soluble phosphorus from the Madison, Wisconsin Nine Springs sewage treatment plant effluent in laboratory studies using ferrous sulfate, ferric sulfate, cupric sulfate, diatomaceous earth, and aluminum sulfate. A dose of 200 mg/1 of aluminum sulfate removed 96-99 percent of the soluble phosphorus in the effluent. A similar dosage of ferrous sulfate, ferric sulfate, and copper sulfate produced the same degree of phosphorus removal but they advised against using copper sulfate as a coagulant because the floc had very poor settling characteristics.

Sawyer (56) has also reported chemical precipitation studies using alum as well as ferric chloride and sodium aluminate on unenriched sewage and on sewage enriched with sodium tripolyphosphate, tetrasodium polyphosphate, and Calgon. Both alum and ferric chloride were found to be highly effective in removing all forms of phosphates but the coagulant requirements were markedly increased by the amount of phosphate present. His results showed that soluble phosphorus could be reduced to less than 1 mg P/1 with 100-130 mg/1 of aluminum sulfate or ferric chloride. Sodium aluminate, however, proved to be an ineffective coagulant unless supplemental caustic was used to raise the pH of the sewage, or alum or sulfuric acid was used to lower the pH to a favorable range.

In preliminary studies on sewage plant effluents with phosphorus concentration of 3.61 to 4.62 mg/1, Curry and Wilson (142) reported that 200 mg/1 of alum removed 96.7 per cent of the phosphates. The treatment was carried out in batches of 4,000 gallons with a mixing time of ten minutes and a 2-hr settling period. Stones (132) obtained an average removal of 94 per cent after 18 hr settling with an alum dose of 400 mg/1 on raw sewage containing 6.25 mg P/1.

Malhotra _et al._ (139) reported alum to be their second choice of coagulant for removing phosphorus from the Nine Springs activated sludge plant at Madison, Wisconsin, in 1964. Their first choice, as mentioned earlier, was lime. Phosphate removal was found to be highly pH dependent, with an optimum pH of 5.75 ± 0.25, and the percentage removal for a given dosage was affected by the phosphate level of the effluent. An alum dose of 250 mg/l was required to remove 94 per cent of the phosphorus in the effluent at the optimum pH (initial phosphorus concentration = 8.3 to 11 mg P/l) but only 78 per cent was removed at pH 8.3. The percentage removal at any alum dose decreased with the increase of phosphorus in the effluent. The addition of Bentonite and Separan NP-10 (Dow Chemical Co.) was found to enhance phosphorus removal but the required dosages were high and thus offset the benefits derived from their use in conjunction with alum.

Operating data for 18 months on the use of alum to remove phosphorus from activated sludge effluent at the South Tahoe Public Utility District treatment plant in California were reported by Slechta and Culp (143). The data showed that the phosphorus in the effluent was reduced from 8.1-9.8 mg P/l to less than 0.33 mg/l, or a reduction of 98 per cent. A method devised at the treatment plant for removing phosphorus using lime was described later by Culp (144). The lime dose was split between the primary sedimentation tank and chemical precipitation tank using reclaimed lime (which already contains some phosphates) in the former and reclaimed lime plus fresh lime make-up in the latter. The sludge from the primary tank is wasted to an incinerator but the relatively low-phosphate sludge from the chemical clarifier is recalcined to recover lime. It was stated that the reuse of lime saved very little in chemical costs but produced a substantial saving by reducing the volume of sludge requiring final disposal by more than half.

Nesbitt (75) reported that lime was found to be the better coagulant, between alum and lime, for removing phosphorus from the sewage plant effluent of State College, Pa. A lime dosage of 330 mg/l and two hr settling removed 91 per cent of the 10.7 mg P/l of total phosphorus. Eliassen (145) tried lime, laboratory alum, liquid alum, ferrous sulfate, ferric chloride, commercial ferric sulfate, and Separan to remove phosphorus from secondary effluent and obtained the best results with liquid alum and ferric sulfate. Removals of 98 per cent were obtained with 300 mg/l of alum or 230 mg/l of ferric sulfate from effluent with a phosphorus content of 7.0 mg P/l.

Neil (146), on the other hand, found a combination of alum and activated silica to show the greatest promise for removing phosphorus from raw sewage, on the basis of calculated costs and efficiency of nutrient removal, from among lime, alum, ferric chloride, and activated silica that were tried singly and in combination. The silica appeared to enhance the action of alum and provide a stronger heavier floc which settled better and was less subject to disintegration. The treatment process was later set up in one of the first plant-scale experiments in the removal of phosphorus from raw sewage.

Thomas (147) described a precipitation method using ferric chloride and similar in effect to activated sludge but useable with or without activated sludge. Phosphorus was precipitated by ferric chloride in a mixing basin with a short detention period and sludge separation was accomplished in a clarifier with an average detention time of 0.5 to 3.0 hrs. The settled sludge was returned to the mixing basin where it was mixed with incoming waste and more ferric chloride. Return sludge was about 6 per cent of the incoming waste flow and ferric chloride additions were about 1 to 1.5 per cent of the ferric chloride in the return sludge. Thomas stated that the process could be carried out simultaneously with the activated sludge process.

Studies on phosphorus removal in integrated or combined chemical precipitation-biological (activated sludge) treatment processes with alum, lime, and other chemicals are summarized in another section of this review.

Ion Exchange

Ion exchange to remove phosphorus from wastes has not been investigated as extensively as chemical precipitation and only a few studies have been reported. Martinez (148) obtained a reduction from 7.5 to 0.11 mg P/1 of the orthophosphate in sewage treatment plant effluent with the resin IRA 410 operated on the chloride cycle. Eliassen _et al_. (149) evaluated several water reclamation treatment processes at Stanford University, Calif., and concluded that ion exchange had the most economical potential for tertiary treatment to recover reuseable water from municipal waste waters. The most promising resin found in the laboratory was a strong base anion exchanger, Duolite A-120D (Diamond Alkali Co.), regenerated with two bed volumes of 10 per cent NaCl solution. This resin removed more than 97 per cent of the phosphates in sand-filtered activated sludge effluent from up to 200 bed volumes in batch experiments and 95 per cent in continuous flow tests. The average phosphorus concentration of the effluents tested was 7.5 mg P/1. In a pilot-plant scale study (150, 151) which was carried out to confirm the laboratory results, removals of phosphorus of 84 per cent was achieved.

Adequate filtration pretreatment of the secondary effluent was found to be important for continuous and trouble-free operation of the ion exchangers.

Pollio and Kunin (152) reported a laboratory study on the renovation of secondary effluent using a modification of a process which was originally developed for the desalination of brackish waters. In the modified process, secondary effluent is treated by an anion exchange resin (Amberlite IRA-68, Rohm and Haas Co.) operated on the bicarbonate cycle which converts the inorganic salts in the effluent to bicarbonate salts. The alkalization step is followed by a flocculation treatment, to remove organic matter, aeration to expel free CO_2, lime softening, and a final dealkalization step using a weak cation exchange resin (Amberlite IRC-84) operated on the hydrogen cycle to further reduce the dissolved salt content and to remove any ammonia which might be present. The anion exchange resin is regenerated with normal aqueous ammonia and the cation resin with normal aqueous acid. Using Haddonfield, N. J., secondary effluent, the process was shown to be able to reduce orthophosphate in the effluent to less than 1 mg/l, as $CaCO_3$, even without the final dealkalinization step. The results also indicated that the anion exchange treatment is sufficient if phosphorus removal is the only goal.

Modifications of the Activated Sludge Process

Increased interest in phosphorus removal has prompted some investigators to reexamine the phosphorus removing capacity of the activated sludge process and attempt to modify its design and/or operation so as to enhance phosphorus removal. As noted previously (cf. p. 55), Jenkins and Menar evaluated phosphorus removal by activated sludge in a pilot plant and found that the phosphorus content of activated sludge, as a fraction of the volatile suspended solids (VSS), was essentially constant and averaged 2.62 per cent of the VSS over a substrate removal rate range of 0.3 to 42.5 lb COD removed/lb VSS/day. From this work, they concluded that the maximum phosphorus removal that can be expected from typical activated sludge plants will not exceed 20 per cent of the influent phosphorus to the aeration tanks. Thus, for a raw sewage containing 10 mg P/1 and 10-15 per cent removal by primary treatment, the maximum overall P removal in an activated sludge plant will be only 28-32 per cent. These workers also tested two modifications of the activated sludge process on a laboratory scale, one a modified contact stabilization system with an additional sedimentation step between stabilization and sludge return, and the other a series high growth-low growth rate system, but found neither of the modifications to show any greater phosphorus removal than a conventionally-operated laboratory activated sludge system.

Some workers have found high phosphorus removals by activated sludge and attributed the phenomenon to metabolic "luxury uptake" by activated sludge microorganisms (153, 77). Levin and Shapiro (153) studied phosphorus uptake by activated sludge in laboratory batch experiments and reported that the sludge organisms are capable of taking up dissolved orthophosphate even in the absence of growth. The rate of aeration and the pH of the mixed liquor were found to have marked effects on the capacity of the organisms to take up phosphate. The uptake of phosphate could be increased by stripping the activated sludge of its phosphate by acidification before returning the sludge to the aeration tank. Based on these studies, these workers developed and patented a modified activated sludge process for enhanced phosphate removal (154). The process, however, was reported to be only moderately successful in the field (155). Further studies of the process and on methods for stripping phosphate from the return sludge were reported by Levin and Shaheen (156). The results of this work were used to develop design criteria and a process schematic for a 1-mgd treatment plant.

Vacker _et al_. (77), observing unusually high phosphorus removals (87-88%) at the San Antonio, Texas, Rilling activated sludge plants while removals at eight other Texas activated sludge plants ranged from only 2 to 55 per cent, investigated the factors affecting the high removals at the San Antonio plants and reported that up to 96 per cent of the total phosphate could be removed when digester supernatant liquors were not returned to the system. A high degree of phosphate uptake was observed to depend on the attainment of high dissolved oxygen levels in the aeration tanks. Analyses of total phosphate content of mixed liquor solids and return sludge at times of high phosphate uptake indicated that as much as 20 to 22 per cent phosphate (as PO_4^{-3}), dry weight basis, were absorbed by the activated sludge. This was interpreted to have been the result of metabolic "luxury uptake" which was propounded earlier by Levin and Shapiro (153). It was pointed out that for maximum phosphate removal by the activated sludge process, a high degree of treatment is necessary but "over treatment", i.e., excessive nitrification and aerobic digestion of solids, is detrimental. The maximum phosphate removal was obtained at an average loading rate of 50 lbs BOD per 100 lbs aeration solids, a sludge volume index of 150, and a dissolved oxygen level in the aeration tanks of about 2.0 mg/l at about midpoint and about 5 mg/l at the effluent end. Modifications in the design and operation of conventional activated sludge plants to improve phosphorus removal were suggested.

To determine the mechanism for the observed high phosphate uptake by activated sludge (77, 153), Menar and Jenkins (155) carried out laboratory and pilot plant studies and concluded that enhanced phosphate removal by activated sludge in hard water sewages, such as that reported for the San Antonio, Texas, Rilling plant, is caused by the precipitation of calcium phosphate followed by enmeshing of the precipitate into the activated sludge floc. The degree of calcium phosphate precipitation is controlled by pH, which in turn is controlled by the CO_2 of the mixed liquor. Enhanced phosphate removal produced sludges with up to 6.8 per cent phosphorus on VSS and up to 10 per cent calcium on VSS, compared to 2-3 per cent phosphorus on VSS and 2 per cent calcium on VSS biologically incorporated into activated sludge. The dissolved oxygen concentration in the mixed liquor was found to have no involvement in the enhanced removal of phosphate by the activated sludge nor in the phosphate release under anoxic conditions and was merely an indicator of the CO_2 content and pH of the mixed liquor. As a result of the studies, an empirical correlation between the apparent solubility product of calcium phosphate and pH was developed which can be used as a predictive tool for determining the required chemical additions to produce a desired dissolved phosphate concentration.

Combined Chemical-Biological Treatment

Because ordinary biological treatment is not efficient in removing phosphorus from waste waters and separate chemical treatment of biologically-treated effluents is expensive, some workers have attempted to develop methods in which chemical and biological treatment are combined or integrated. Tenney and Stumm (157, 158) investigated a combined biological-chemical process in which chemical flocculation was substituted for the bioflocculation of the microorganisms produced in the biological stage. They reasoned that if chemical flocculation could be used for the flocculation of microorganisms, which normally occur by bioflocculation in the aeration tanks, the design of the biological stage can be optimized without giving consideration to the bioflocculation phase. Thus, it will be possible to reduce significantly the detention time of the biological (aeration) stage and operate it as a "high-rate, low solids retention unit." The additional cost resulting from the chemical treatment can be compensated for partially or fully by a reduction in the cost of biological treatment resulting from shortened detention times. Phosphorus will be removed simultaneously in the chemical precipitation stage and the effluent from such a system will therefore promote neither heterotrophic activity (oxygen depletion) nor autotrophic activity (algal growth) in receiving waters.

In batch flocculation experiments using biologically-treated synthetic substrate from both batch and continuous-flow activated sludge systems, Tenney and Stumm found that the dispersed microorganisms behave like hydrophilic colloids and could be readily flocculated by synthetic organic polyelectrolytes and the metal coagulants Al(III) and Fe(III). The polyelectrolytes, however, were ineffective in removing phosphates. Good results were found to depend on the pH and degree of agitation, the optimum pH range being around 5 to 6 for Al(III) and 4.5 to 5.5 for Fe(III). A stoichiometric relationship was found to exist between the concentration of chemicals required for flocculation and the concentration of microorganisms and phosphates.

Barth and Ettinger (159), on the other hand, proposed adding the phosphate precipitant directly to the aeration basin and reported a study using a 100-gpd conventional activated sludge pilot plant carried out to evaluate the process. In a preliminary screening of various chemicals on raw sewage, the highest phosphorus removals were obtained with a combination of aluminum sulfate and lime (30 mg Al/l + 20 mg Ca/l), which removed 90 per cent of the phosphorus. Without any chemical additive only 40 per cent of the phosphorus was removed. The effectiveness of the alum plus lime treatment was verified in the pilot plant. Experiments using sodium aluminate were also conducted and it was found that it was as effective as the alum-lime mixture. A dosage of 10 mg/l of aluminum added as $NaAl(OH)_4$ removed 90-98 per cent of the influent phosphorus and did not interfere with nitrification or carbon and solids removal. The sludge produced was reported to have better settling characteristics than either a biological sludge or aluminum hydroxide floc alone and also digested satisfactorily anaerobically.

Eberhardt and Nesbitt (160, 161) evaluated under continuous bench-scale operations several combined chemical-biological systems using aluminum sulfate and high-rate activated sludge with both synthetic and domestic sewage and reported that essentially complete removal of phosphorus could be attained. The phosphorus removal efficiency was related to the pH and the Al:P (aluminum to influent phosphorus) molar ratio, an Al:P ratio of 1.5 to 2.1 being necessary to effect almost complete removal. Aluminum sulfate as high as 335 mg/l added to the aeration basin did not adversely affect the biological performance of the activated sludge. The weight of sludge to be expected from the combined treatment was estimated to be over twice as much as that from a conventional activated sludge plant but the volume would be about the same.

Barth et al. (162) described a three-stage combined chemical-biological process designed to remove not only phosphorus but also nitrogen, COD, and suspended solids from primary effluent. The process consists of a high-rate activated sludge system, nitrification, and denitrification integrated into a single continuous-flow treatment plant. Each of the stages has its own recycle system in order that enriched cultures of microorganisms can develop. Sodium aluminate is added to the aeration basin to precipitate phosphorus which is removed via the waste sludge. The aerator effluent, which contains the residual carbonaceous matter and the majority of the influent nitrogenous material, then flows to the nitrification section. The nitrified effluent then goes to the denitrification stage where methyl alcohol is added to force biological denitrification to occur. Flash aeration is provided at the mixed liquor outlet of the denitrification stage to scrub nitrogen gas bubbles and to furnish dissolved oxygen in the final effluent. To insure low phosphorus levels in the final effluent, a polish dose of sodium aluminate is added just before the flash aerator in the denitrification stage.

Thomas (147) has described a chemical precipitation system for phosphate removal using ferric chloride and recirculation of the ferrous phosphate sludge formed. The process is claimed to be useable with or without activated sludge. The coagulant could be added either before or after the activated sludge basin. The method in combination with activated sludge has been applied at the treatment plants at Mannedorf and Uster (163, 164, 165). The process was found to be stable and could remove more than 90 per cent of the total phosphorus in the settled sewage. Recirculation of the ferrous phosphate sludge reduced the amount of iron needed by one-half to two-thirds of the quantity needed were the ferric chloride to be used only once. The total volume of sludge was not increased, the sludge produced was easily dewatered, and there was no release of phosphate from the sludge during anaerobic digestion. Thomas reported that chemical precipitation with aluminum sulfate proved to be more expensive and to produce greater quantities of sludge than with ferric chloride.

Combined systems in which phosphorus precipitation is accomplished before biological treatment, instead of in or after the aeration basin, have been investigated by Buzzell and Sawyer (15), Schmid and McKinney (166, 167), and Albertson and Sherwood (168, 169, 170). Buzzell and Sawyer (15) treated raw sewage with lime and passed the effluent, after settling and with or without recarbonation, through bench-scale continuous flow, completely mixed activated sludge units operated with theoretical detention periods of six hours or less. The lime treatment removed 80 to 90 per cent of the total phosphorus in the raw sewage but the activated sludge treatment was unsuccessful owing to the low BOD of the waste water after the lime treatment.

Successful operation in the laboratory of a process consisting of lime treatment to remove the bulk of the phosphorus in raw sewage and activated sludge to remove the remaining phosphorus was reported by Schmid and McKinney (166, 167). A lime dosage of 150 mg/l $Ca(OH)_2$ added to raw sewage produced a pH of 9.5, phosphate removal of 80 per cent, BOD removal of 60 per cent, and suspended solids removal of 90 per cent. The laboratory activated sludge units, which were operated as high-rate completely mixed systems, were found to operate satisfactorily on the lime-treated sewage with no deterioration in either sludge or effluent quality.

Albertson and Sherwood (168, 169, 170) described a system similar to those described above, except that a portion of the chemical sludge is recirculated to the primary clarifier. Lime is added to the primary clarifier to raise the pH to 9.5-10.0 and precipitate phosphorus, followed by activated sludge treatment. The lime treatment is reported to remove 85 per cent of the suspended solids, 60-70 per cent of the BOD, and 80-85 per cent of the phosphorus. Sufficient primary sludge to maintain a high suspended solids concentration (500-2000 mg/l) in the primary clarifier is recirculated and additional lime is added to maintain the pH at the desired level. The lime addition can be automatically controlled and will vary depending on the raw sewage flow, alkalinity, and desired phosphorus removal. The waste primary sludge and waste activated sludge are treated in a solids treatment system designed for lime recovery. The waste sludge produced by the system is claimed to be easy to dewater and less expensive to handle and treat or dispose than conventional sludge, because of the predominance of primary sludge.

Algal Systems

Previous studies on algal systems in phosphorus removal have been summarized earlier. (cf. pp. 2-9)

Effluent Spraying

Spraying on land is one of the oldest methods of disposal of raw or treated sewage but fell into uncommon use as more efficient mechanical and chemical treatment processes were developed. A few applications and studies of the method for nutrient control and water reclamation have been reported.

To protect the recreational lakes in the area from nutrients, spray irrigation of secondary effluent from the city of Detroit Lakes, Minn. was employed (171). The effluent amounting in volume to 0.74 mgd was applied to a 4-acre tract through a sprinkler irrigation system at an average daily rate of 6.8 inches per day. After a 4-year period, it was found that the ground water level in the area had risen by 3 feet and the total phosphorus in the ground water increased from 0.6 to 2.9 mg P/l. Because of the high rate of irrigation, vegetation did not grow in the sprinkled area during the summer months. It was concluded that if normal vegetation were to be grown, five feet of irrigation per year would probably be the maximum allowable, considering the limited growing season in the region. This rate would require an area of approximately 200 acres. It was pointed out that such an extensive system would be expensive and difficult to operate during the winter months.

Foster et al. (172) studied the effectiveness of hillside spraying of treatment plant effluent in keeping nutrients out of Lake Tahoe, California. From analyses of samples of effluents applied to and samples of seepage and surface runoff from the spray area, tney concluded that intermittent spraying of primary or secondary effluent effectively reduced the sewage nutrients below the level that could be obtained by conventional treatment processes alone. Phosphate removals varying from 76 to 93 per cent were obtained during periods of normal operation and loadings. The removal efficiency, however, dropped when the soil mantle became soaked with rainfall or snow melt during the early spring months. The poorest performance was observed when the hillside was frozen and one area was sprayed without rest. The best results were obtained when one section was sprayed for 2 days and allowed to rest for 4 days, at an average dosing rate of about 40,000 gallons per acre per day. To obtain the best performance, they recommend that spray facilities should be planned to permit intermittent application even during severe winter periods.

Pennypacker et al. (173) investigated the renovation of sewage effluent by irrigation of forested areas and found that the phosphorus in the applied effluent could be almost completely removed by the upper 6 inches of soil. The secondary effluent used had an average phosphorus concentration of 8.5 mg P/1 during the experimental period and was applied at rates varying from 1 to 4 inches per week for 23 weeks.

Other Methods

A process called 'Electrolytic Sewage Purification' developed by Føyn at Oslo, Norway, has been described by Rohlich (174). The process uses a divided container equipped with electrodes connected to the negative and positive poles of a battery. One portion of the chamber contains sea water and the other contains sewage mixed with approximately 10 to 15 per cent sea water. Chlorine is developed at the graphite anode and hydrogen and alkali in the chamber containing the iron cathode. Thus, the chemical conditions necessary for the precipitation of phosphorus are created. The phosphate is adsorbed on magnesium hydroxide floc and is floated to the surface by the hydrogen bubbles that are formed.

One disadvantage of anaerobic digestion of sewage sludge is the production of supernatant liquid rich in the nutrients originally present in the sludge. If the supernatant liquid is returned to the treatment plant, as is commonly practiced, much of the nutrient content is discharged in the plant effluent. To avoid this problem, Irgens and Halvorson (175) proposed aerobic treatment of the sludge and investigated the method in the laboratory. They found that when the sludge was treated aerobically, a significant portion of the carbonaceous matter was oxidized to carbon dioxide and water and the rest assimilated into microbial protoplasm. The process tied up all the available nitrogen and phosphorus so that practically none remained dissolved in the suspending liquid. It is doubtful whether this process will find wide application because of the high cost and duration of aeration involved. The process requires a detention period of about 20 days.

CONTINUOUS CULTURE SYSTEMS

It is widely recognized that the study of the kinetics of microbial growth is conveniently and best made with the use of continuous culture systems. A continuous culture system, as opposed to a batch culture, consists of a culture of microorganisms in some sort of vessel into which fresh substrate or medium is continuously or semi-continuously added and mixing can be applied. The microorganisms and their products and by-products and unreacted materials emerge from the culture vessel at the same rate as the feed. The volume of the culture is maintained constant by an overflow or other suitable device. Continuous culture systems have long been used in the cultivation of microorganisms. During the last two decades there has been a revival of interest and increase in the application of such systems in biological research and the production of microorganisms and/or their products for experimental or other purposes. The use of continuous cultures has greatly facilitated the study and improved the understanding of various aspects of microbial growth.

The basic continuous culture system and the one in common use in microbiological work consists of a single culture vessel or reactor whose contents are well stirred so that the incoming medium is instantaneously and uniformly dispersed throughout and the effluent has the same composition as the reactor contents. This type of system can be controlled in two ways, namely, (a) by controlling the input flow rate of the culture medium, and (b) by controlling the organism concentration or density. In the first method, the flow rate of the feed is held constant below a certain value and the organism concentration adjusts itself to a rate which is determined by the rate of supply of a limiting nutrient. This device was named 'Bactogen' by Monod (176) and 'Chemostat' by Novick and Szilard (177, 178) and various other names by others (179).

In the second method of control, the density of organisms is maintained at some fixed level by some sensing and measuring device, such as a photocell, which also regulates the flow rate of the medium. The density measuring device increases the flow rate of the medium when the culture density increases and decreases it when the density falls. A system operated in this manner is commonly known as a 'turbidostat'. Novick (180) referred to these two types of systems as 'externally controlled' and 'internally controlled', respectively.

Theory of Continuous Cultures

The principles underlying the theoretical treatment of continuous culture systems (chemostat type) were first laid down by Monod (176) and Novick and Szilard (177, 178) and have been discussed and expanded by Herbert *et al*. (181), Herbert (182), Novick (180), and other workers (179, 183-186). The theory involves the growth rate of microorganisms, relationship between growth rate and concentration of substrate, and relationship between growth rate and substrate consumption. Rate or kinetic equations for a continuous culture system can be developed by applying these relationships to balance equations of the system.

Organism Growth Rate. The growth rate of microorganisms in a continuous culture is logarithmic and can be expressed by the familiar 'exponential growth' equation, or

$$\frac{dx}{dt} = \mu x \qquad (1)$$

where

x = organism concentration, mass/volume
t = time
μ = specific growth rate, time^{-1}

According to Equation 1, the growth rate of organisms is proportional to the concentration of organisms present. The equation involves the assumption that all the organisms are viable, i.e., capable of reproducing, or at least only an insignificant fraction are not capable of reproduction. Monod (187) has stated that this is a fairly good assumption for homogeneous populations. If this assumption does not hold, the equation must be modified to include an expression for the loss of cells by death or other causes and the organism concentration used should be that of the viable organisms. In sanitary engineering, the effect on the growth rate of the loss of cells by death and endogenous respiration is taken into consideration by introducing a term, k_d, the specific decay rate, in Equation 1 (188).

$$\frac{dx}{dt} = (\mu - k_d)x \qquad (2)$$

The doubling time, t_d, which is the time required for the mass to double, is frequently used to characterize the behavior of a microbial culture. The doubling time is related to the specific growth rate, μ, according to the equation

$$t_d = \frac{\log_e 2}{\mu} \tag{3}$$

Relationship between Growth Rate and Nutrient Concentration. The relationship between the growth rate and the concentration of the limiting growth factor is an important aspect of continuous cultures. From his studies using pure bacterial cultures and simple substrates, Monod (187, 189) noted that this relationship could be described by a hyperbolic function similar to the Michaelis-Menten equation for enzyme substrate interaction. Monod's equation is given below and a plot of the equation is shown in Fig. 2.

$$\mu = \hat{\mu} \frac{S}{K_s + S} \tag{4}$$

where

$\hat{\mu}$ = the maximum specific growth rate, time^{-1}

S = concentration of limiting nutrient, mass/volume

K_s = a 'saturation constant' numerically equal to the nutrient concentration at which the growth rate is one-half the maximum value (i.e., $\mu = \hat{\mu}/2$), mass/volume.

From Figure 2 it can be seen that the specific growth rate is approximately proportional to the substrate concentration, S,

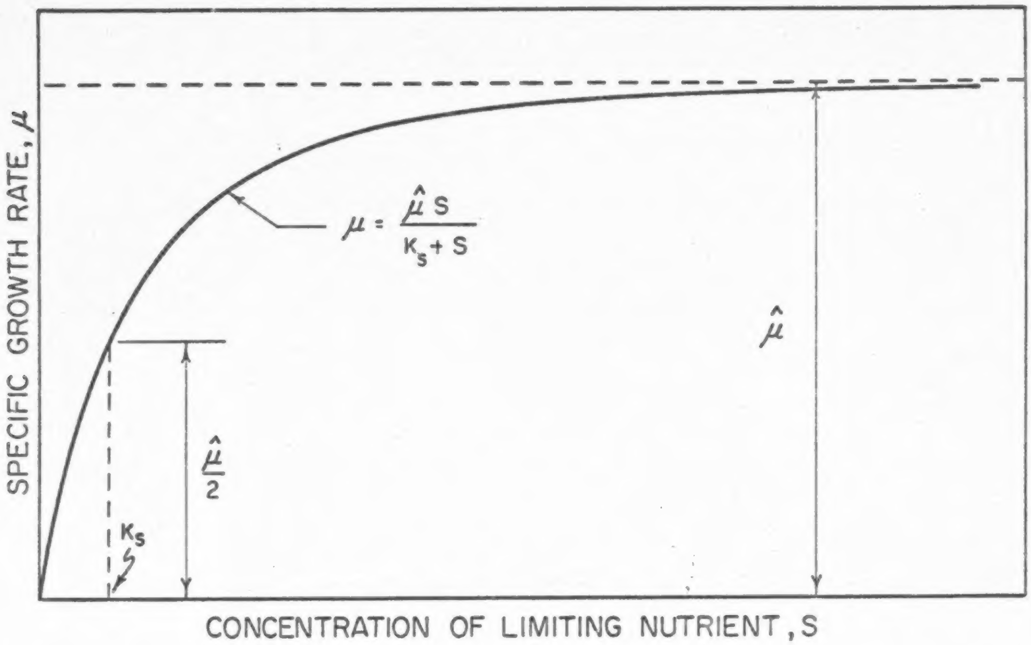

FIGURE 2. RELATIONSHIP BETWEEN GROWTH RATE AND LIMITING NUTRIENT CONCENTRATION

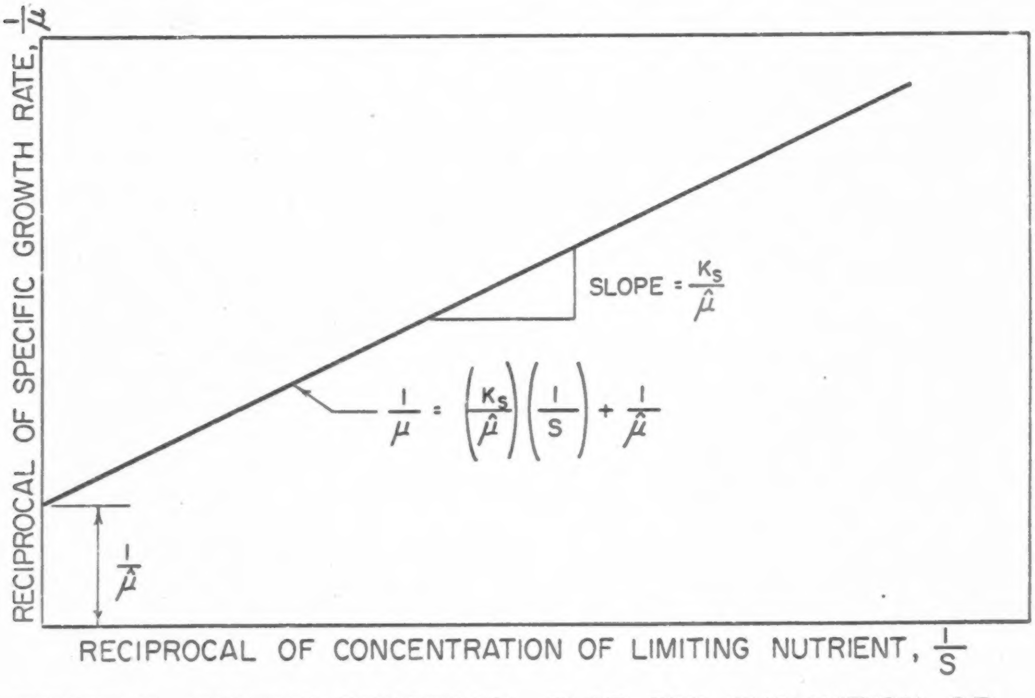

FIGURE 3. DOUBLE RECIPROCAL PLOT FOR EVALUATION OF $\hat{\mu}$ AND K_s

when this is small but tends to maximum value, $\hat{\mu}$, as the substrate concentration increases. The value of K_s is usually small. In the case of bacteria, James (179) reported that *M. tuberculosis*, *E. coli*, *Bacillus subtilis*, and *Salmonella typhimurium* with glucose as the limiting substrate showed K_s values from 20 mg to 2 mg per liter and *E. coli* strains with limiting tryptophane showed values in the range of 10^{-3} µg/ml.

A wide variety of substances can act or be used as the controlling growth factor. Novick (180) has mentioned (a) a required amino acid (tryptophane, arginine, proline, histidine), (b) the energy source (lactate, glucose, maltose), (c) the nitrogen source, (d) phosphate, and (e) sulfate as among the controlling growth factors that have been employed. Environmental factors such as the temperature, pH value, aeration, etc. can also markedly influence the growth rates. Pipes (190, 191) showed that light and carbon dioxide can serve as the growth-limiting factor in algal cultures.

Various other expressions have been developed for the growth rate as a function of the substrate concentration but none of these has had the wide acceptance of the equation of Monod. One such expression is that of Moser (192):

$$\mu = \hat{\mu} \frac{S^n}{K_s + S^n} \tag{5}$$

Moser's function is similar to that of Monod except that the exponent of the substrate concentration is not unity. When the exponent is unity, the expression reduces to that of Monod.

Another expression is that which has been proposed by Teissier (193). The equation has the following form:

$$\frac{d\mu}{dS} = C(\hat{\mu} - u) \qquad (6)$$

or in its integrated form:

$$\mu = \hat{\mu}\,(1 - e^{-CS}) \qquad (7)$$

where $C = 1/K_s$ = a constant. Schulze and Lipe (194) and Schulze (195, 196), in studies of the growth of _E. coli_ on glucose, found Teissier's equation to fit their data better than the Monod equation. Shelef _et al._ (197), in studies of algal growth with nitrogen as the limiting nutrient, used a modified Teissier equation with the following form:

$$\mu = \hat{\mu}\,(1 - e^{-S/K_A}) \qquad (8)$$

in which K_A is a constant equal to the value of S at a specific growth rate of 0.632 $\hat{\mu}$ instead of 0.5 $\hat{\mu}$ in the original Teissier equation.

Contois (198) believed that the specific growth rate is a function of population density, P, as well as the concentration of the limiting nutrient, S. From his studies and those of others, he derived the following model for bacterial growth:

$$\mu = \frac{\mu_m S}{BP + S} \qquad (9)$$

where μ_m and B are growth parameters that are constants under defined conditions.

It can be seen from Figure 2 that the value of $\hat{\mu}$ cannot be determined accurately by plotting the values of μ as a function of S because the value of $\hat{\mu}$ is the limit of an asymptote of the curve as shown in the figure. However, Monod's equation can be transformed into a straight line form by taking the reciprocals of both sides of the equation, as shown in Equation 10 and Figure 3, and the values of $\hat{\mu}$ and K_s can be determined graphically.

$$\frac{1}{\mu} = \left(\frac{K_s}{\hat{\mu}}\right)\left(\frac{1}{S}\right) + \frac{1}{\hat{\mu}} \qquad (10)$$

As can be seen from Figure 3, the slope of the plot of the reciprocal form of Monod's equation is $K_s/\hat{\mu}$ and the y-intercept is $1/\hat{\mu}$.

Cell Yield. The cell yield relates the amount of cells synthesized to the amount of substrate or nutrient consumed and is usually expressed in terms of a yield factor or coefficient, Y, defined as

$$Y = \frac{\text{weight of cells produced}}{\text{weight of nutrient consumed}} \qquad (11)$$

The value of Y has been found by Monod (189) and others (178, 181) to be relatively constant for a given organism and given substrate. Other workers, however, have found the yield coefficient to vary with the dilution rate. A model described by a varying yield coefficient is described later on.

The yield coefficient can also be expressed as a differential, as has been done by Moser (192):

$$\frac{dx}{dt} = -Y \frac{dS}{dt} \qquad (12)$$

or

$$Y = - \frac{dx}{dt} \qquad (13)$$

If both sides of Equation 12 are multiplied by 1/x,

$$\frac{1}{x}\frac{dx}{dt} = -Y\frac{dS}{dt}\frac{1}{x} \quad (14)$$

or

$$\mu = YK \quad (15)$$

where

K = specific transfer rate, time^{-1}

Mathematical Analysis of a Single-State Continuous Culture System

All continuous culture problems involving completely-mixed reactors can be treated by writing balance equations for cells, substrates, products, etc., in which the overall rate of change in concentration of any component is given as the resultant of the rates of all the processes tending to increase or decrease it. For a single-stage reactor (chemostat type) without cell recycle and neglecting organism decay, the balance equation for cells is (individual terms refer to rates in each case):

$$\text{Change in cell concentration} = \text{Inflow} + \text{Growth} - \text{Outflow}$$

or

$$V\frac{dx_1}{dt} = Fx_0 + \mu x_1 V - Fx_1 \quad (16)$$

in which

x_0 = cell concentration in the feed, mass/volume

x_1 = cell concentration in the reactor and effluent, mass/volume

F = flow rate of the feed, volume/time

V = culture volume

In the case when there are no cells in the inflow to the reactor, i.e., $x_0 = 0$,

$$\frac{dx_1}{dt} = \mu x_1 - Dx_1 \qquad (17)$$

where

$D = \frac{F}{V}$ the dilution rate or the flow rate of substrate per unit volume of culture, time^{-1}

Equation 17 may be stated as the change in cell concentration with respect to time is the increase by growth, μx_1, minus the loss by dilution or washout, Dx_1.

At steady-state, there will be no change in the cell concentration, i.e., $dx_1/dt = 0$, and therefore

$$\mu = D \qquad (18)$$

or the growth rate in the steady state is equal to the dilution rate. This relationship is an important characteristic of the single-stage completely mixed constant volume reactor.

The balance equation for the substrate or nutrient is:

Change in substrate concentration = Inflow - Outflow - Consumption

or

$$V\frac{dS_1}{dt} = FS_0 - FS_1 - \frac{\mu x_1 V}{Y} \qquad (19)$$

in which

S_0 = the substrate concentration in the feed, mass/volume

S_1 = the substrate concentration in the reactor and effluent, mass/volume

Writing dS/dt = 0 for the steady state condition results in

$$\frac{\mu x_1}{Y} = D(S_0 - S_1) \qquad (20)$$

and since u = D from Equation 18,

$$x_1 = Y(S_0 - S_1) \qquad (21)$$

Substituting Equation 4 in Equation 18 and solving for S_1, the following equation is obtained:

$$S_1 = K_s \frac{D}{\hat{\mu} - D} \qquad (22)$$

The parameters $\hat{\mu}$, K_s, and Y are kinetic 'constants' and once they have been determined for a particular organism-substrate combination, the steady-state values of the organism and substrate concentrations in the reactor and in the effluent can be predicted for any value of dilution rate and influent substrate concentration. These relationships are shown graphically in Figure 4 for the values of the growth 'constants' and substrate concentration in the feed indicated in the figure.

Figure 4 shows that over a wide range of dilution rate, the steady state value of S_1 is very low, i.e., almost all the added substrate is consumed. Only at dilution rates close to a critical value (designated D_c in Figure 4) will unused substrate appear in the culture. The critical dilution rate has been designated as the

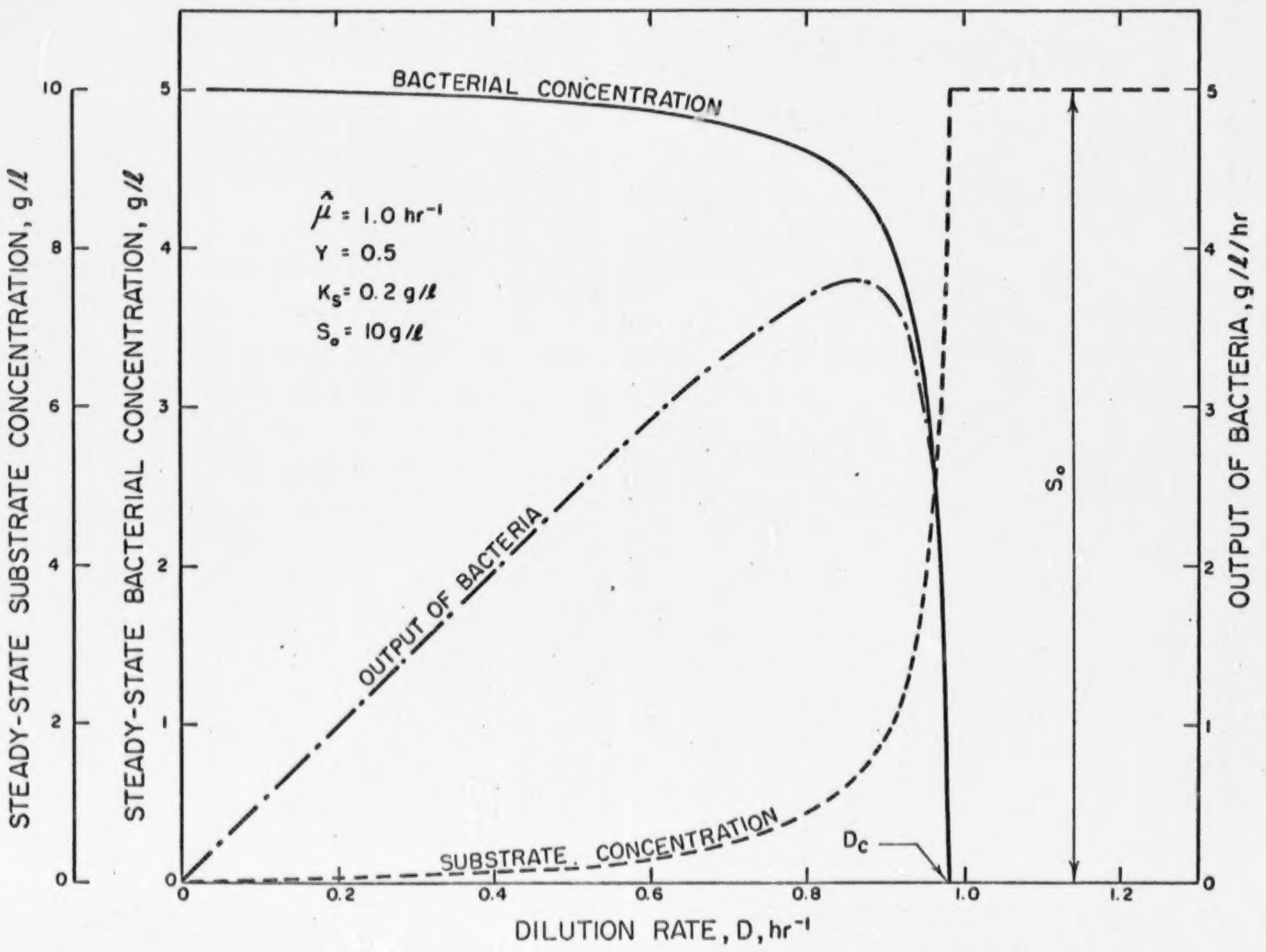

FIGURE 4. STEADY-STATE RELATIONSHIPS IN A SINGLE-STAGE CONTINUOUS BACTERIAL CULTURE (182)

"washout point" by Herbert (183). At dilution rates above the washout point the concentration of organisms in the culture will be zero and the substrate concentration will be equal to that of the feed, since there will be no substrate consumption.

The balance equation for organisms may also be written to include the effect of organism decay, as follows:

$$\text{Change in cell concentration} = \text{Inflow} + \text{Growth} - \text{Decay} - \text{Outflow}$$

or

$$V\frac{dx_1}{dt} = Fx_0 + \mu x_1 V - k_d x_1 V - Fx_1 \tag{23}$$

in which

k_d = the specific organism decay rate, time^{-1}

In this case, again assuming no cells in the incoming feed, the dilution rate at steady-state will no longer be equal to the specific growth rate μ but is given by the following equation

$$D = \mu - k_d \tag{24}$$

The corresponding equations for the steady-state concentration of organisms and substrate can be developed in a manner similar to that used above and are:

$$x_1 = \frac{YD(S_0 - S_1)}{(D + k_d)} \tag{25}$$

$$S_1 = \frac{K_s(D + k_d)}{\hat{\mu} - (D + k_d)} \tag{26}$$

The single-stage system without cell recycle and organism decay was the first to be treated mathematically by Monod (176) and Novick and Szilard (177, 178) and later in more detail by Herbert *et al*. (181). Mathematical models for other systems, such as the single-stage reactor with cell recycle, two-stage systems, etc., can be developed using the same principles employed above. Some of these systems or modifications have been discussed in some detail by Herbert (182) and Andrews (188).

Model with a Varying Yield Coefficient

The model for the single-stage continuous culture system developed above is described by a constant yield coefficient, Y. Although the early workers have found the yield coefficient to be a constant in their studies, other workers have reported a varying yield coefficient. Marr *et al*. (199) realized that the yield coefficient may not be a constant even for pure cultures utilizing a single organic compound and attributed this to the effect of a maintenance requirement for exogenous substrate by the culture. Hetling *et al*. (200) found the yield coefficient to change with the dilution rate in continuous-flow studies using pure bacterial cultures. They explained the variation as being due to maintenance requirement and presence of dead cells in the system. Schulze (195) reported a varying yield coefficient in his studies on the activated sludge process with pure cultures of *E. coli* and glucose as substrate. He attributed this to the effect of endogenous respiration. Mor and Fiechter (201, 202) also found the yield to vary with the dilution rate for *Saccharomyces cerevisiae* grown on ethanol. The cell yield was low at small dilution rates, rose to a maximum, and then decreased again. They explained the behavior at the low dilution rates as being due to the effect of a constant endogenous metabolism and increased inhibitor formation and concentration. A constant endogenous metabolism becomes a larger fraction of the total metabolism as the latter is decreased, i.e., a relative increase of energy consumption for maintenance at lower growth rates takes place. Herbert _et al_. (181) also noted that the yield coefficient decreased with an increasing dilution rate and remarked that in their theoretical treatment they assumed that the yield coefficient was independent of the dilution rate and that this assumption was probably not valid. The assumption of a constant yield coefficient is probably reasonable only for limited ranges of the dilution rate for certain organism-substrate combinations.

A model described by a varying yield coefficient due to the influence of maintenance energy has been described by Koga and Humphrey (203). The constant yield coefficient is defined as

$$-\frac{dS}{dt} = \frac{1}{Y}\frac{dx}{dt} \qquad (27)$$

The effect of maintenance energy required independently of the growth rate was taken into consideration by using a respiration coefficient R as follows:

$$-\frac{dS}{dt} = \frac{1}{Y}\frac{dx}{dt} + Rx \qquad (28)$$

The observed or overall yield coefficient, Y_o, is then given by

$$Y_o = -\frac{dx/dt}{dS/dt} = \frac{dx/dt}{(1/Y)(dx/dt) + Rx}$$

which can be expressed in the form

$$\frac{1}{Y_o} = \frac{1}{Y} + R\frac{x}{(dx/dt)} = \frac{1}{Y} + \frac{R}{\mu} \qquad (29)$$

Since μ is a function of S, the overal yield coefficient, Y_o, is now a variable as indicated in Equation 29 instead of being a constant.

The basic equations of the system with a variable yield coefficient are:

$$\frac{dx}{dt} = x\left(\frac{\hat{\mu}S}{K_s + S} - D\right) \qquad (30)$$

$$\frac{dS}{dt} = D(S_0 - S) - \frac{x}{Y}\frac{\hat{\mu}S}{K_s + S} - Rx \qquad (31)$$

The steady state solutions to Equations 30 and 31 are:

$$x = \frac{DY(S_0 - S)}{D + RY} \qquad (32)$$

$$S = \frac{K_s D}{\hat{\mu} - D} \qquad (33)$$

For comparison, it is recalled that the steady-state solutions to the system with a constant yield coefficient are:

$$x = Y(S_0 - S) \qquad (21)$$

$$S = \frac{K_s D}{\hat{\mu} - D}$$

Van Uden (204) has also derived an equation relating the yield coefficient, Y, to the specific growth rate, μ, when Y varies due to energy requirements for maintenance. When the energy source is the limiting nutrient, he assumed that the specific transfer rate, K, in Equation 15 is the sum of two specific rates, one for growth only, K_g, and the other for maintenance only, K_m. Thus,

$$\mu = YK \tag{15}$$

$$= Y(K_g + K_m) \tag{34}$$

Assuming that the specific growth rate is a linear function of the specific uptake rate of limiting nutrient used for growth only,

$$\mu = \hat{Y} K_g \tag{35}$$

where

$\hat{Y}$ = the 'maximum yield factor', the limit of Y as K_m tends to zero

Combining Equations 34 and 35 results in

$$Y = \hat{Y} \frac{K_g}{K_g + K_m} \tag{36}$$

which may be written as

$$Y = \hat{Y} \frac{u}{\hat{Y}K_m + u} \tag{37}$$

or

$$Y = \hat{Y} \frac{u}{A + u} \tag{38}$$

where

$$A = \hat{Y}K_m \tag{39}$$

Adding Equations 35 and 39

$$u + A = \hat{Y}K \tag{40}$$

The parameter A is referred to as the "specific maintenance rate" and K_m the "maintenance coefficient."

The following relation can be shown to apply in the chemostat

$$\mu x + Ax = \hat{Y}(S_0 - S)D \tag{41}$$

from which it follows that

$$Y = \hat{Y}\frac{D}{D + A} \tag{42}$$

and a double reciprocal plot of Y against D will be acceptably linear when $\hat{Y}$ and A are invariant with respect to D or nearly so.

$$\frac{1}{Y} = \frac{1}{\hat{Y}} \quad \frac{A}{\hat{Y}}\frac{1}{D} \tag{43}$$

CHAPTER III

EXPERIMENTAL APPARATUS AND PROCEDURES

DESCRIPTION OF APPARATUS

In the experimental phase of this investigation, laboratory algal growth units were operated as continuous cultures (chemostats). The apparatus employed was the algal growth system which was developed and described by Oswald (205). Figure 5 shows a schematic diagram of the apparatus.

The culture vessel or reactor is a cylindrical Pyrex-glass tube approximately 2¼ in. in diameter and 28 in. long. Its maximum working volume is about one liter. The culture tube is mounted vertically on aluminum racks attached to one wall of a wooden housing. The unit is fed with nutrient medium through a horizontal side arm at the bottom of the culture tube and effluent flows by gravity into an effluent collector through two horizontal overflows near the top of the tube. The unit is illuminated by a bank of four 30-watt, 36-in. long, "Optima" (Duro-Test Co., New Bergen, N. J.) fluorescent daylight lamps arranged vertically and in parallel along the back wall of the wooden housing. A battery of six growth units was used in this study.

Each growth unit is fed automatically at 30-minute intervals by a system consisting of a feed reservoir, siphon, solenoid valve, measuring flask, injection pump, electrodes, timer and vacuum tube

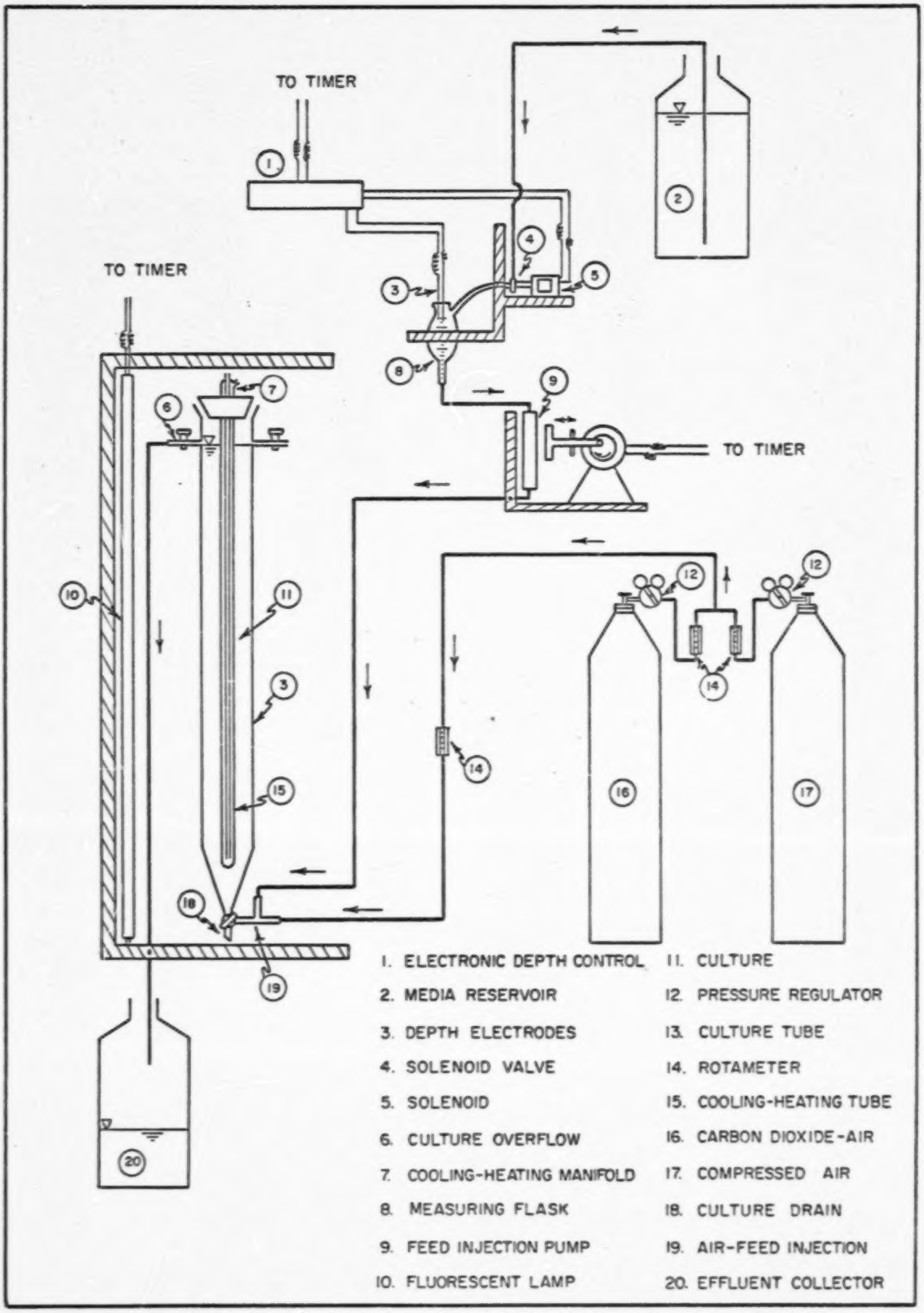
TO TIMER
TO TIMER
TO TIMER
1
2
3
4
5
6
7
8
9
10
11
3
15
14
14
12
12
16
17
18
19
20
1. ELECTRONIC DEPTH CONTROL
2. MEDIA RESERVOIR
3. DEPTH ELECTRODES
4. SOLENOID VALVE
5. SOLENOID
6. CULTURE OVERFLOW
7. COOLING-HEATING MANIFOLD
8. MEASURING FLASK
9. FEED INJECTION PUMP
10. FLUORESCENT LAMP
11. CULTURE
12. PRESSURE REGULATOR
13. CULTURE TUBE
14. ROTAMETER
15. COOLING-HEATING TUBE
16. CARBON DIOXIDE-AIR
17. COMPRESSED AIR
18. CULTURE DRAIN
19. AIR-FEED INJECTION
20. EFFLUENT COLLECTOR

FIGURE 5. SCHEMATIC DIAGRAM OF CHEMOSTATIC ALGAL GROWTH UNIT

relay, and connecting rubber tubing. The feed reservoir is a 10- or 20-liter Pyrex bottle connected to the measuring flask through the solenoid valve, by means of a siphon made from glass tubing and a piece of rubber tubing. The measuring flask is a 100-ml centrifuge tube with a re-entrant inlet at its side and an outlet at the bottom.

The feeding operation is controlled by a Cramer cycling timer which makes two complete revolutions per hour. Cams attached to the timer activate microswitches that control the opening of the solenoid valve and the operation of the feed pump. At the appropriate time, the solenoid valve opens allowing nutrient medium to flow by gravity from the feed reservoir into the measuring flask. In the measuring flask are two electrodes and when these are contacted by the rising liquid level, a vacuum tube circuit is completed causing the solenoid valve to close. The settling of the electrodes, which can be varied, determines the volume of nutrient solution to be delivered during each feeding time. After a short interval the vacuum tube relay linking the solenoid to the electrodes is inactivated by the timer to prevent refilling of the measuring flask until the next feeding time.

Following the filling of the measuring flask with the desired amount of medium, another timer-operated microswitch turns on the feed pump and the nutrient solution in the flask is then injected under pressure into the culture tube. The nutrient solution enters the culture tube at the horizontal nutrient-air inlet at the bottom of the tube and is mixed thoroughly with the culture by the rising bubbles of air in the tube. The feed pump used was a Brosites proportioning pump (Andrew Technical Service, Chicago, Ill.). The duration of pump operation is also controlled by the timer and can be adjusted so that all the medium in the measuring flask is fed to the unit.

Aeration and mixing of the cultures were effected by bubbling air enriched with CO_2. This enriched-air supply was blended from a cylinder of compressed air and a cylinder of compressed 95% air-5% CO_2 in a proportion of one part to one to four parts of 95% air-5% CO_2, thus obtaining a CO_2 concentration in the mixture of one to 2.5 per cent. The flow rates from the cylinders were measured and controlled with rotameters. The enriched-air supply line leads to a distribution manifold from which individual air lines go to the growth units. Air was supplied to each unit at a rate of about 0.2 cu ft per hr. This rate was sufficient to mix the culture thoroughly and provide an adequate supply of carbon dioxide. Individual rotameters on the air line to each unit permit control and adjustment of the air flow rate to the units.

A constant culture temperature is maintained by continuously circulating cooling or heating water through a glass heat exchanger in the form of a cooling tube extending the length of the culture tube. This cooling tube is connected to a distribution manifold from a thermostatically-controlled water cooling-heating system. The water enters an inner tube of the glass heat exchanger and flows up through an annulus into a collection manifold of return piping to the water source. The temperature of the culture could be held within $\pm$ 0.5°C. with this system.

TEST ORGANISMS

Two strains of Chlorella pyrenoidosa, the Emerson strain and TX 71105, were employed as the test organism. The majority of the experiments, however, were made with the TX 71105. The Emerson strain is a low-temperature strain with optimum temperature for growth of about 25°C. TX 71105 was developed by Sorokin and co-workers (206). It is a thermotolerant strain which grows best at a temperature of 39°C. Pure cultures of the test organism were obtained from the Chemical Biodynamics Laboratory of the University of California, where stock cultures are maintained in agar slants under bacteria-free conditions and sub-cultured at monthly intervals to maintain their genetic characteristics.

COMPOSITION OF THE MEDIUM

In order to eliminate the composition of the culture medium, except for its phosphorus concentration, as a variable factor, a synthetic culture medium was used in the study. The composition of the medium used is shown in Table VI and VII. The components of the medium are similar to those of the modified urea medium which was developed at the Sanitary Engineering Research Laboratory of the University of California (207). With respect to plant nutrients it closely simulates oxidized sewage. The modified urea medium is a modification of the nitrate medium which was developed by Myers (197). Sodium carbonate solution was added to provide alkalinity and a 0.025 N H_2SO_4 solution was used for pH adjustment.

The culture medium was prepared in 10- or 20-liter acid-washed Pyrex bottles. The phosphorus concentration of the medium

TABLE VI

COMPOSITION OF THE CULTURE MEDIUM

Nutrient	Preparation	
	Stock solution moles/liter	Amount ml/liter
Urea	2.5	5.0
KH_2PO_4	0.01-0.10	Varies
K_2HPO_4	0.01-0.10	Varies
$MgSO_4 \cdot 7H_2O$	1.0	5.0
Fe Versenol 120	0.1	0.87
Micronutrients	See Table VII	0.5
$CaCl_2$	0.01	0.0

TABLE VII

FORMULA OF THE MICRONUTRIENT STOCK SOLUTION (207)

Nutrient	grams/liter
H_3BO_3	2.86
$MnCl_2 \cdot 4H_2O$	1.81
$ZnSO_4 \cdot 7H_2O$	0.222
$CuSO_4 \cdot 5H_2O$	0.079
MoO_3 (99.5%)	0.015
$CoCl_2 \cdot 6H_2O$	0.040

was checked from time to time during the course of each experiment. The medium was not sterilized and after initiation of a chemostat run, no attempt was made to maintain bacteria-free cultures.

EXPERIMENTAL PROCEDURE

All parts of the apparatus which would come in contact with culture medium and algal culture were thoroughly washed with a dilute acid solution (1:10 HCl) and rinsed several times with distilled water. After assembly, each culture tube was filled with nutrient medium and inoculated with algae from a culture which had been previously developed using algae from an agar slant. A volume of 1 to 5 ml was used as inoculum for each culture unit. The culture units were then operated as batch cultures under continuous 24-hr illumination to allow the cultures to develop. When actively-growing cultures were manifested, usually within one or two days, feeding was begun and the cultures allowed to grow until steady state was reached. Steady state was considered to have been established when the concentrations of algae and phosphorus in the culture had become essentially constant. The length of time for a culture to reach steady stage ranged from 5 to 10 days, depending on the phosphorus level in the feed and the dilution rate. The cultures were sampled and the algal and phosphorus concentrations determined frequently during the course of each experiment. When the cultures were at steady state, sampling was done daily. The pH and temperature were also checked frequently. The effluent volume was measured daily to check the feed flow rate and adjust it if necessary.

The cultures were allowed to remain at steady state for at at least three days or three detention periods, whichever was longer, before an experimental run was terminated or the dilution rate changed for another run. A series of experiments with a given feed phosphorus concentration was usually started with an experimental run at a low dilution rate.

Adherence of algal growth to the culture tube wall occurred occasionally. When this condition was observed, the culture tube was immediately cleaned or replaced.

SAMPLING AND LABORATORY MEASUREMENTS

Culture samples were collected by opening a three-way stopcock at the bottom of the culture tube and withdrawing the sample into an acid-washed Erlenmeyer flask. The volume of sample withdrawn ranged from 50 to 100 ml. The pH was measured immediately after sample collection, using a small volume of sample, with a Leeds and Northrup Model No. 7047 pH meter.

Algal concentration was measured in two ways: (a) optical density, and (b) volatile suspended solids concentration. The optical density determinations were used only as a rapid means of observing the behavior of the culture and its progress towards steady state. The optical density was measured with a Bausch and Lomb Spectronic 20 spectrophotometer, using a whole or diluted sample, with a 1-in. tube and a wavelength of 675 mu.

The volatile suspended solids concentration was determined by filtering a measured volume of sample through a tared GFC glass fiber filter and weighing the filter after drying at 105°C., and after ignition at 600°C. followed by cooling. The filtrate was saved and used for the residual medium phosphate determination. The total soluble phosphorus was determined by the stannous chloride procedure described in the "Standard Methods for the Examination of Water and Waste Water, 12th edition" (208).

CHAPTER IV

RESULTS AND DISCUSSION

EXPERIMENTS WITH C. PYRENOIDOSA 71105

A number of experiments at various phosphorus levels in the feed and at different dilution rates were carried out with the 71105 strain of *Chlorella pyrenoidosa*. The phosphorus-level in the feed used ranged from 0.5 to 15 mg P/1 and the dilution rate from 0.5 to 4 per day. Data recorded during the experiments are given in Table VIII.

Evaluation of Kinetic Parameters

The relationship between the growth rate and the concentration of phosphorus is shown in Figures 6 to 11 for each set of experiments with a given phosphorus concentration in the incoming medium. In each plot, the dilution rate was used to represent the specific growth rate. Theoretical curves of the equation proposed by Monod, using values of the maximum specific growth rate, $\hat{\mu}$, and saturation constant, K_s, determined from the data, are also shown in the figures. It can be seen from the figures that there is fair agreement between the theoretical curves and the experimental data.

The applicability of the Monod equation for describing the data was evaluated by plotting the reciprocals of the specific growth rate and the concentration of phosphorus. The plots are given in Figures 12 to 19 and they show that the data can be reasonably well

TABLE VIII
SUMMARY OF GROWTH AND KINETIC DATA - EXPERIMENTS WITH *C. pyrenoidosa* 71105

Expt. No.	Dilution Rate D day^{-1}	Detention Period θ days	Phosphorus Main Feed S_o mg P/1	Phosphorus in Culture S_1 mg P/1	Algal Concentration x_1 mg/1	$\frac{1}{D}\left(- \frac{1}{\mu}\right)$	$\frac{1}{S_1}$	Cell Y Yield Y $\frac{\text{mg cells}}{\text{mg P}}$	Phosphorus Content of Cell Mass % of Dry Weight	Algal Production P mg/l/day	Efficiency of Phosphorus Removal %
A-I-1	0.50	2.00	0.521	0.007	192	2.00	143	374	0.268	96	98.5
A-I-2	0.65	1.54	0.521	0.011	183	1.54	91	359	0.279	119	97.8
A-I-3	1.02	0.98	0.521	0.015	141	0.98	66.6	278	0.359	144	97.1
A-I-4	1.51	0.662	0.505	0.039	92	0.662	25.7	197	0.507	139	92.3
A-I-5	1.95	0.513	0.510	0.043	58	0.513	23.8	124	0.806	113	91.7
A-II-1	0.49	2.04	1.00	0.008	363	2.04	125	366	0.274	178	99.2
A-II-2	0.65	1.54	1.00	0.009	325	1.54	111	328	0.305	211	99.1
A-II-3	0.98	1.02	1.00	0.020	230	1.02	50	235	0.426	226	98.0
A-II-4	1.30	0.77	0.99	0.022	165	0.77	45.5	170	0.59	213	97.6
A-II-5	1.50	0.667	0.99	0.040	142	0.667	25	150	0.669	212	96.0
A-II-6	1.97	0.507	1.00	0.067	105	0.507	14.9	113	0.888	207	93.3
A-II-7	3.98	0.252	1.01	0.120	47	0.252	8.33	53	1.895	187	88.2
A-III-1	0.52	1.92	2.02	0.009	690	1.92	111	344	0.292	360	99.5
A-III-2	0.656	1.53	2.02	0.010	615	1.53	100	306	0.327	401	99.5
A-III-3	1.01	0.99	2.01	0.018	441	0.99	55.5	222	0.451	446	99.3
A-III-4	1.50	0.667	1.98	0.046	312	0.667	21.7	161	0.62	468	97.9
A-III-5	2.02	0.495	2.01	0.050	190	0.495	20	97	1.031	384	97.5
A-III-6	4.00	0.25	1.97	0.220	98	0.25	4.55	56	1.785	392	88.7
A-IV-1	0.51	1.96	3.48	0.010	760	1.96	100	219	0.456	388	99.6
A-IV-2	1.02	0.98	3.48	0.034	628	0.98	29.4	182	0.549	640	98.9
A-IV-3	1.49	0.67	3.50	0.029	465	0.67	34.4	134	0.745	694	99.2
A-IV-4	2.05	0.49	3.49	0.058	336	0.49	17.3	98	1.02	685	98.2
A-IV-5	3.02	0.33	3.50	0.150	245	0.33	6.67	73	1.37	743	95.7
A-IV-6	4.00	0.25	3.50	0.460	195	0.25	2.18	64	1.56	760	86.6
A-V-1	1.01	0.99	5.03	0.021	792	0.99	47.6	158	0.631	800	99.5
A-V-2	1.51	0.66	5.03	0.048	602	0.66	20.8	121	0.826	912	99.1
A-V-3	1.98	0.505	4.98	0.056	470	0.505	17.9	96	1.049	930	99.0
A-V-4	3.05	0.34	4.95	0.221	318	0.34	4.55	67	1.488	934	95.5
A-V-5	3.98	0.252	4.95	0.649	239	0.252	1.54	56	1.8	956	86.7
A-VI-1	1.02	0.98	7.00	0.029	830	0.98	34.5	119	0.84	846	99.6
A-VI-2	1.50	0.667	7.00	0.051	685	0.667	19.6	99	1.011	1028	99.2
A-VI-3	2.02	0.495	6.98	0.061	560	0.495	16.4	81	1.236	1130	99.2
A-VI-4	3.01	0.332	7.03	0.320	435	0.332	3.12	65	1.543	1310	99.5
A-VI-5	4.01	0.249	7.01	0.841	317	0.249	1.19	52	1.94	1275	88
A-VII-1	1.00	1.00	9.84	0.71	855	1.00	1.41	94	1.07	855	92.8
A-VII-2	1.55	0.645	9.97	1.45	718	0.645	0.69	84	1.19	1115	85.4
A-VII-3	2.03	0.493	10.05	1.75	575	0.493	0.57	69	1.44	1159	82.6
A-VII-4	3.01	0.332	10.10	3.94	490	0.332	0.254	79	1.26	1475	61.0
A-VII-5	3.97	0.252	9.96	4.55	342	0.252	0.220	63	1.58	1360	54.3
A-VIII-1	1.01	0.99	14.97	5.63	896	0.99	0.178	96	1.04	905	62.3
A-VIII-2	1.51	0.66	14.97	6.45	740	0.66	0.155	86	1.15	1117	57.0
A-VIII-3	2.00	0.50	14.92	6.56	628	0.50	0.152	75	1.33	1255	56.0
A-VIII-4	2.98	0.336	14.99	7.12	510	0.336	0.141	65	1.55	1520	52.5
A-VIII-5	4.02	0.248	14.95	7.28	385	0.248	0.138	53	1.81	1545	51.3

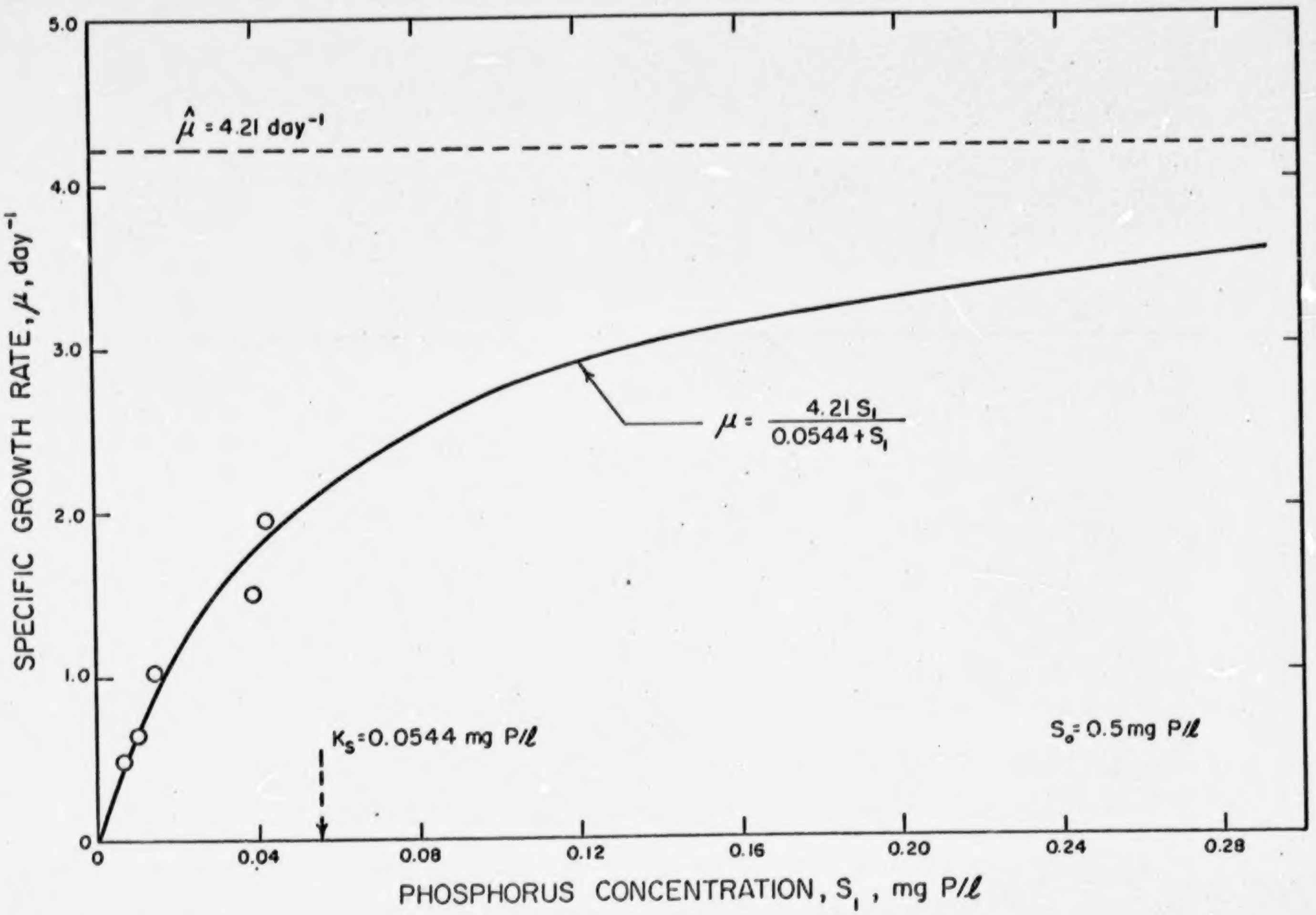

FIGURE 6. GROWTH RATE AS A FUNCTION OF PHOSPHORUS CONCENTRATION (C. Pyrenoidosa 71105; T = 39 ± 0.5°C; pH = 7.5 ± 0.1)

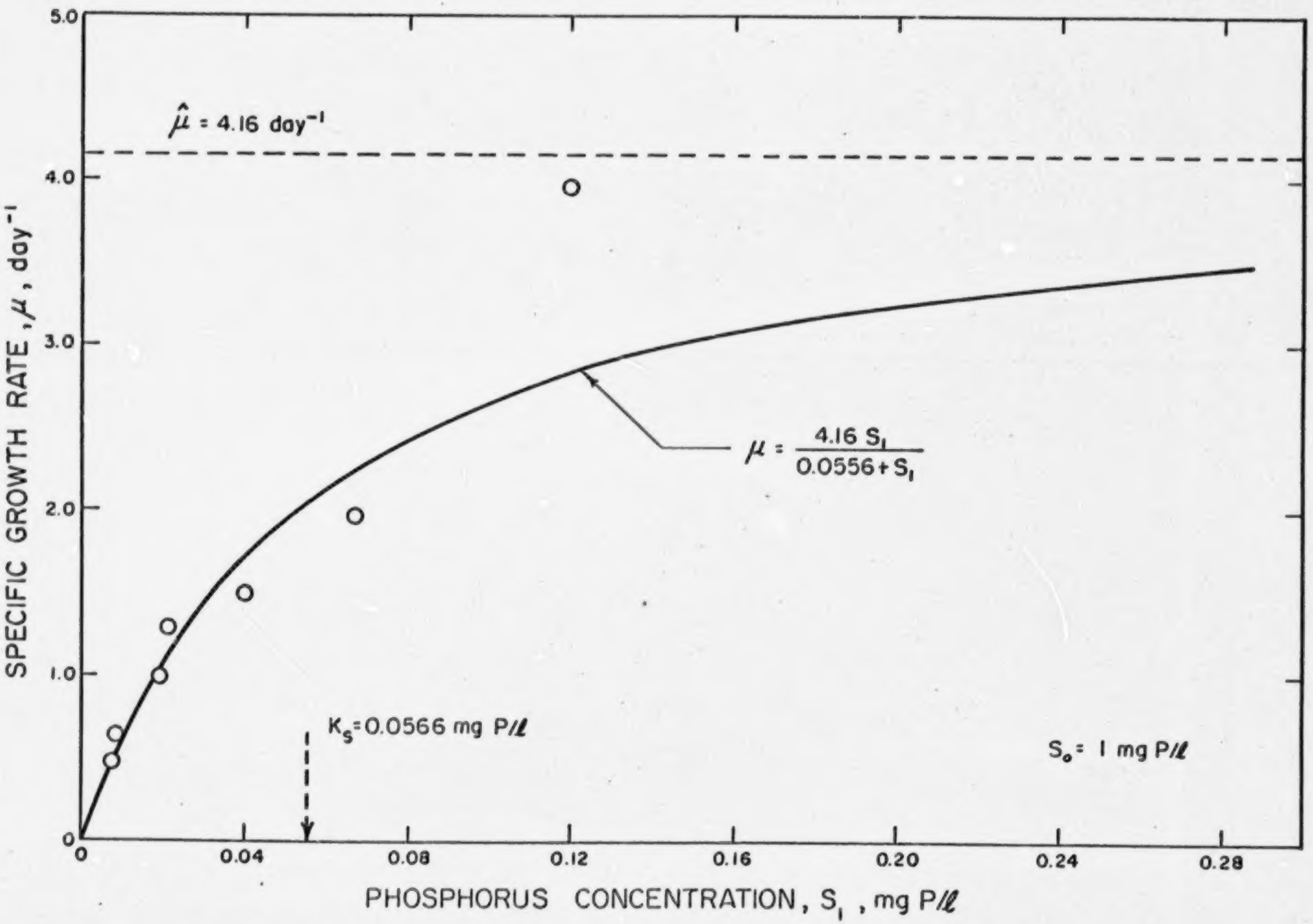

FIGURE 7. GROWTH RATE AS A FUNCTION OF PHOSPHORUS CONCENTRATION (C. Pyrenoidosa 71105; T = 39 ± 0.5 °C; pH = 7.5 ± 0.1)

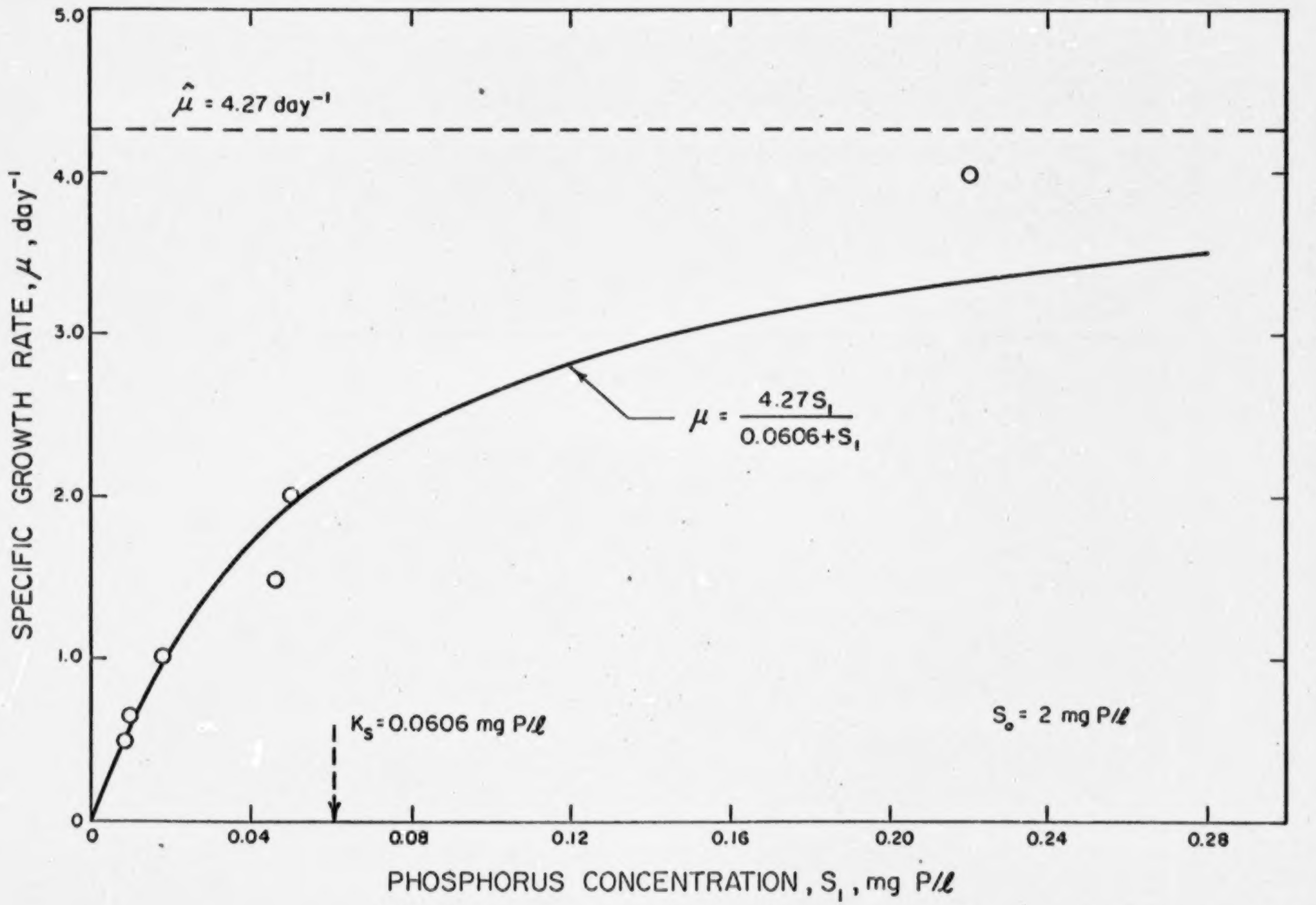

FIGURE 8. GROWTH RATE AS A FUNCTION OF PHOSPHORUS CONCENTRATION (C. Pyrenoidosa 71105; T = 39 ± 0.5 °C; pH = 7.5 ± 0.1)

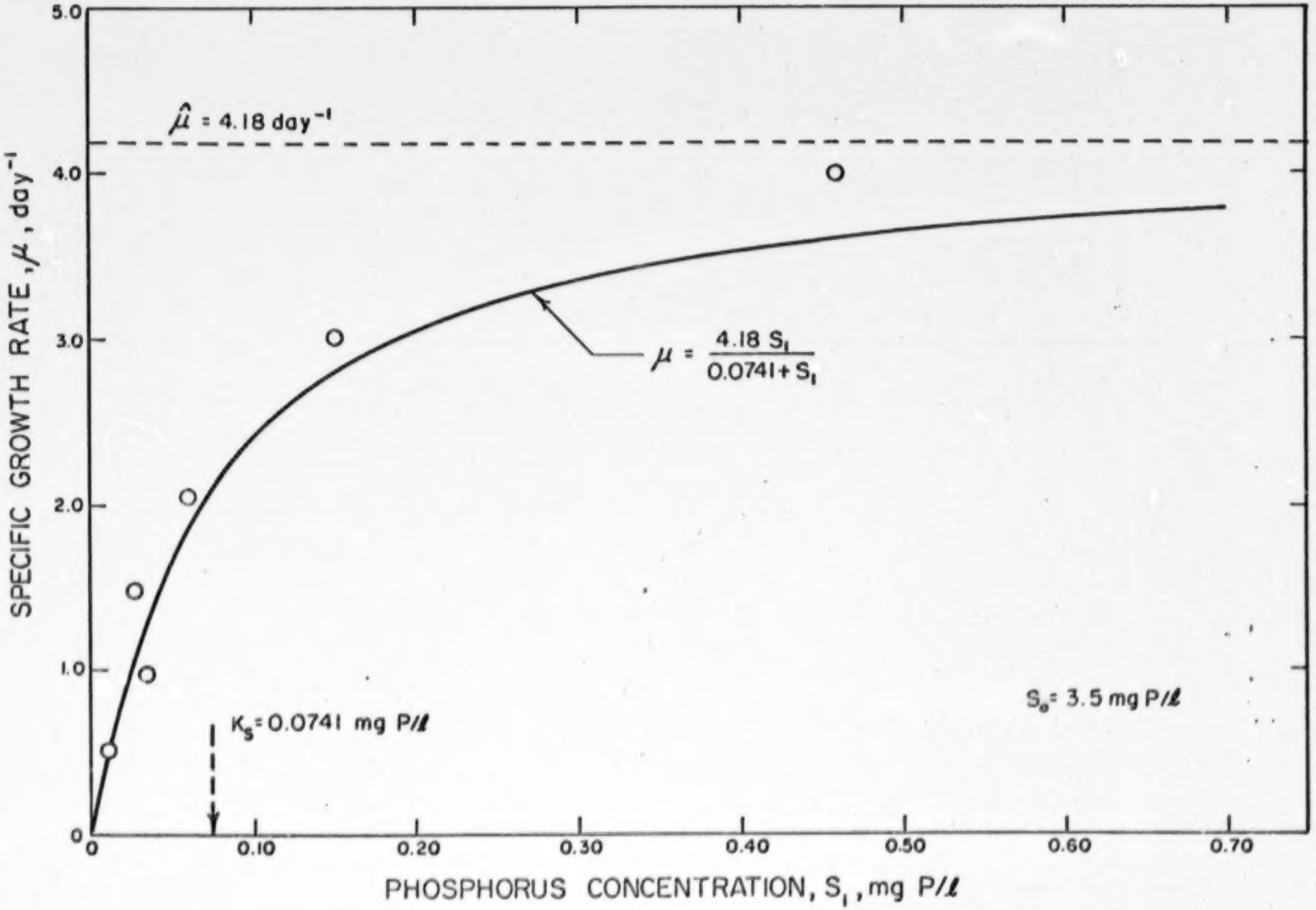

FIGURE 9. GROWTH RATE AS A FUNCTION OF PHOSPHORUS CONCENTRATION
(C. Pyrenoidosa 71105; T = 39 ± 0.5 °C; pH = 7.5 ± 0.1)

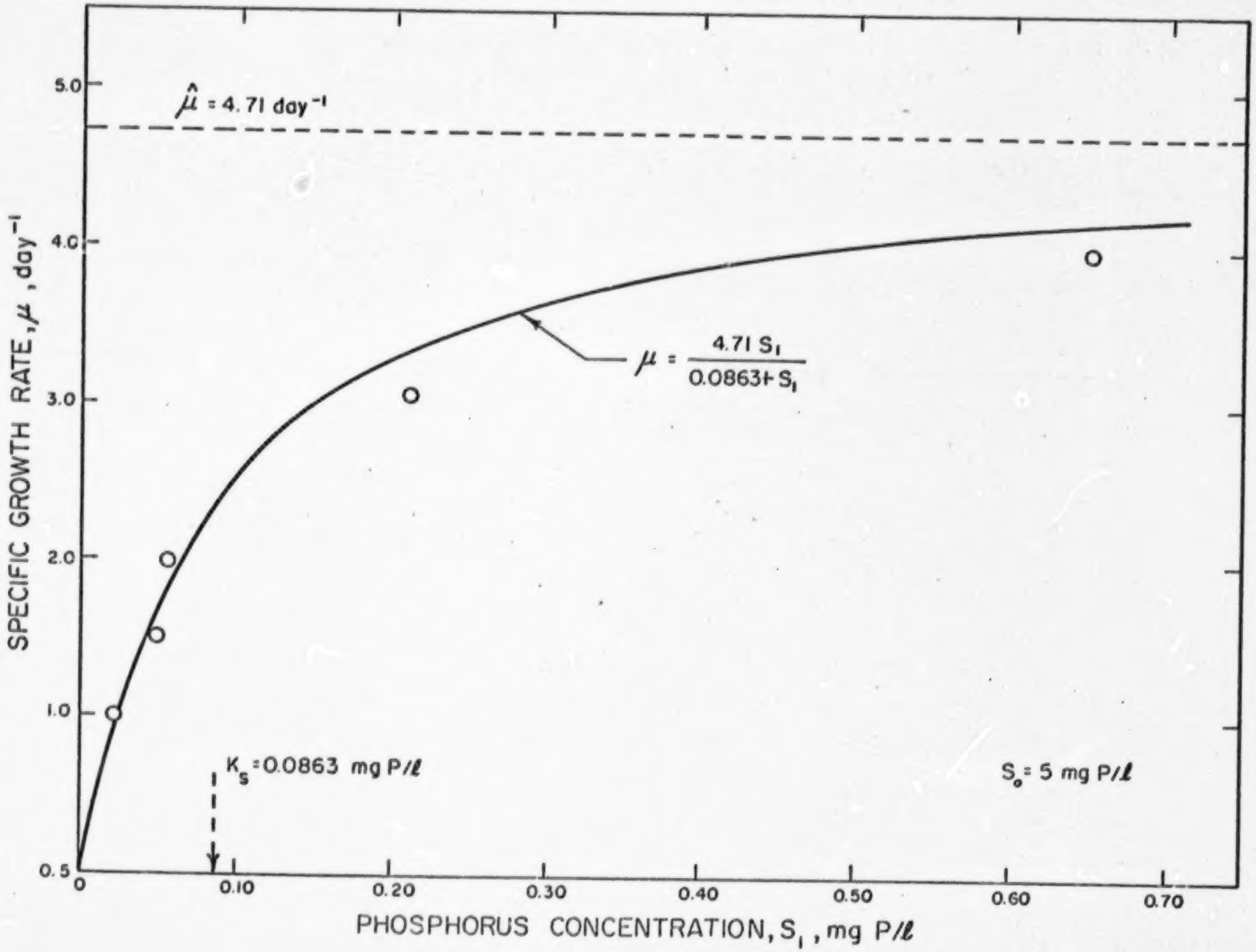

FIGURE 10. GROWTH RATE AS A FUNCTION OF PHOSPHORUS CONCENTRATION (C. Pyrenoidosa 71105; T= 39 ± 0.5°C ; pH= 7.5 ± 0.1)

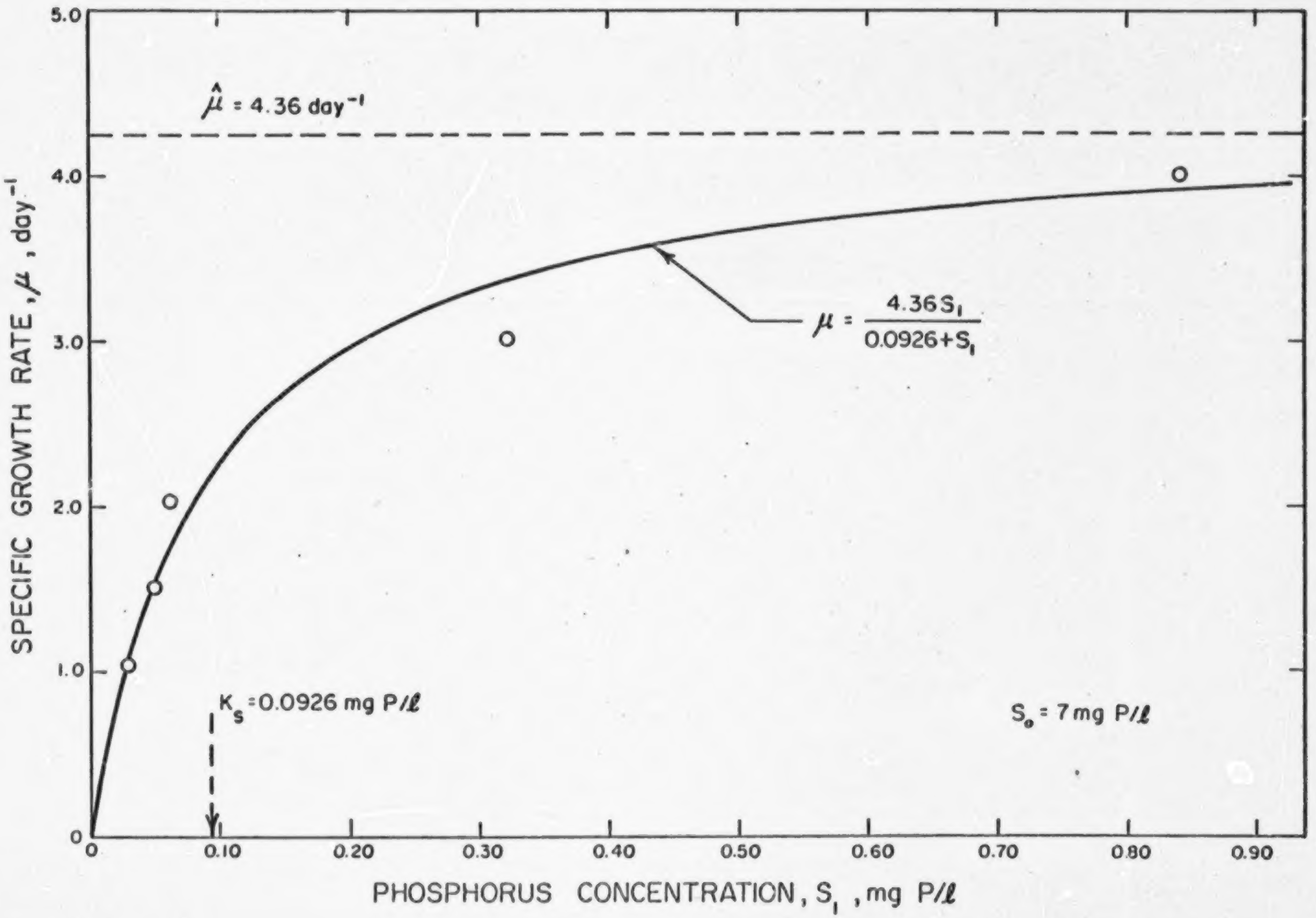

FIGURE 11. GROWTH RATE AS A FUNCTION OF PHOSPHORUS CONCENTRATION
(C. Pyrenoidosa 71105; T = 39 ± 0.5 °C; pH = 7.5 ± 0.1)

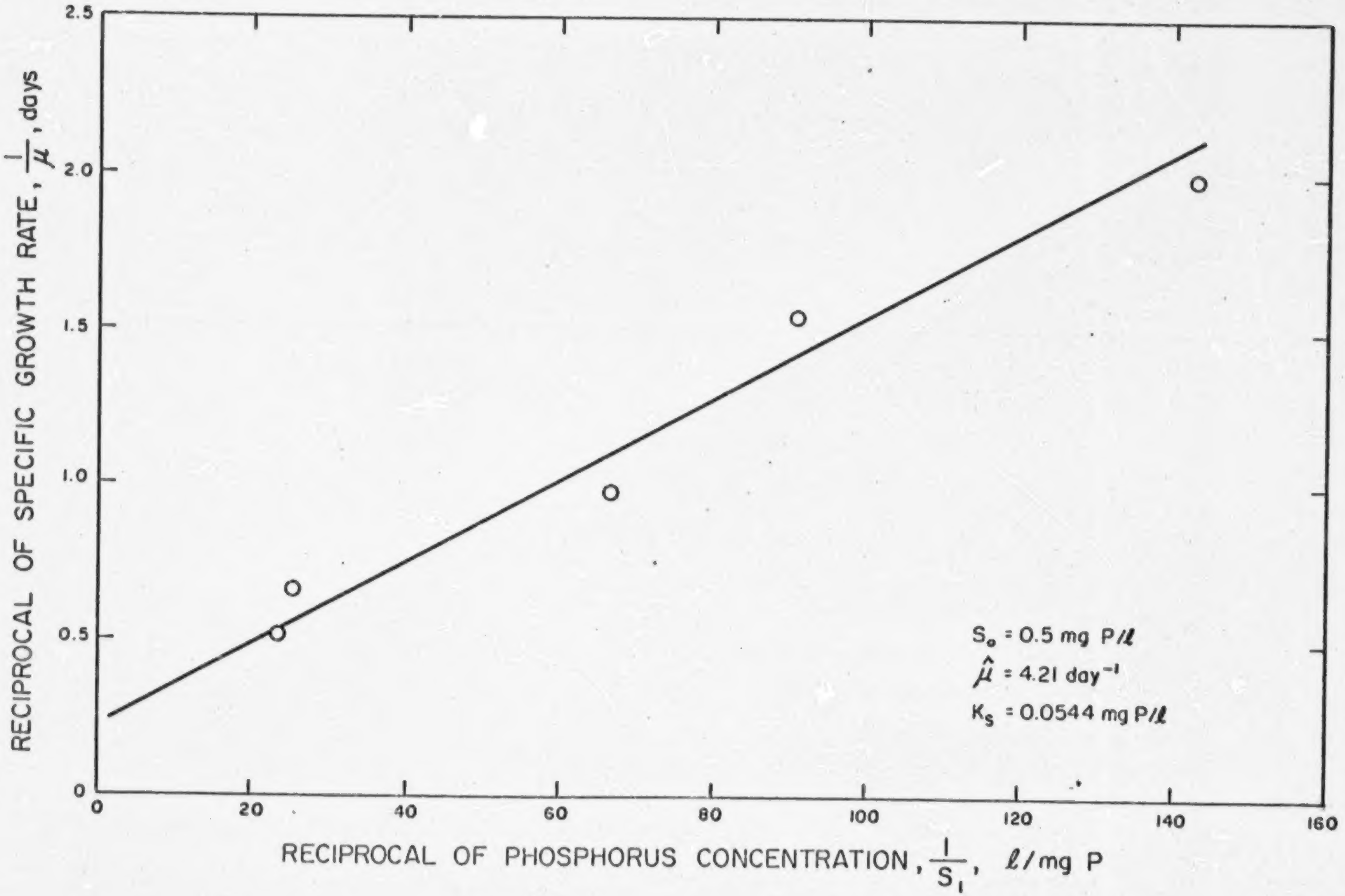

FIGURE 12. DETERMINATION OF THE MAXIMUM SPECIFIC GROWTH RATE, $\hat{\mu}$, AND SATURATION CONSTANT, K_s

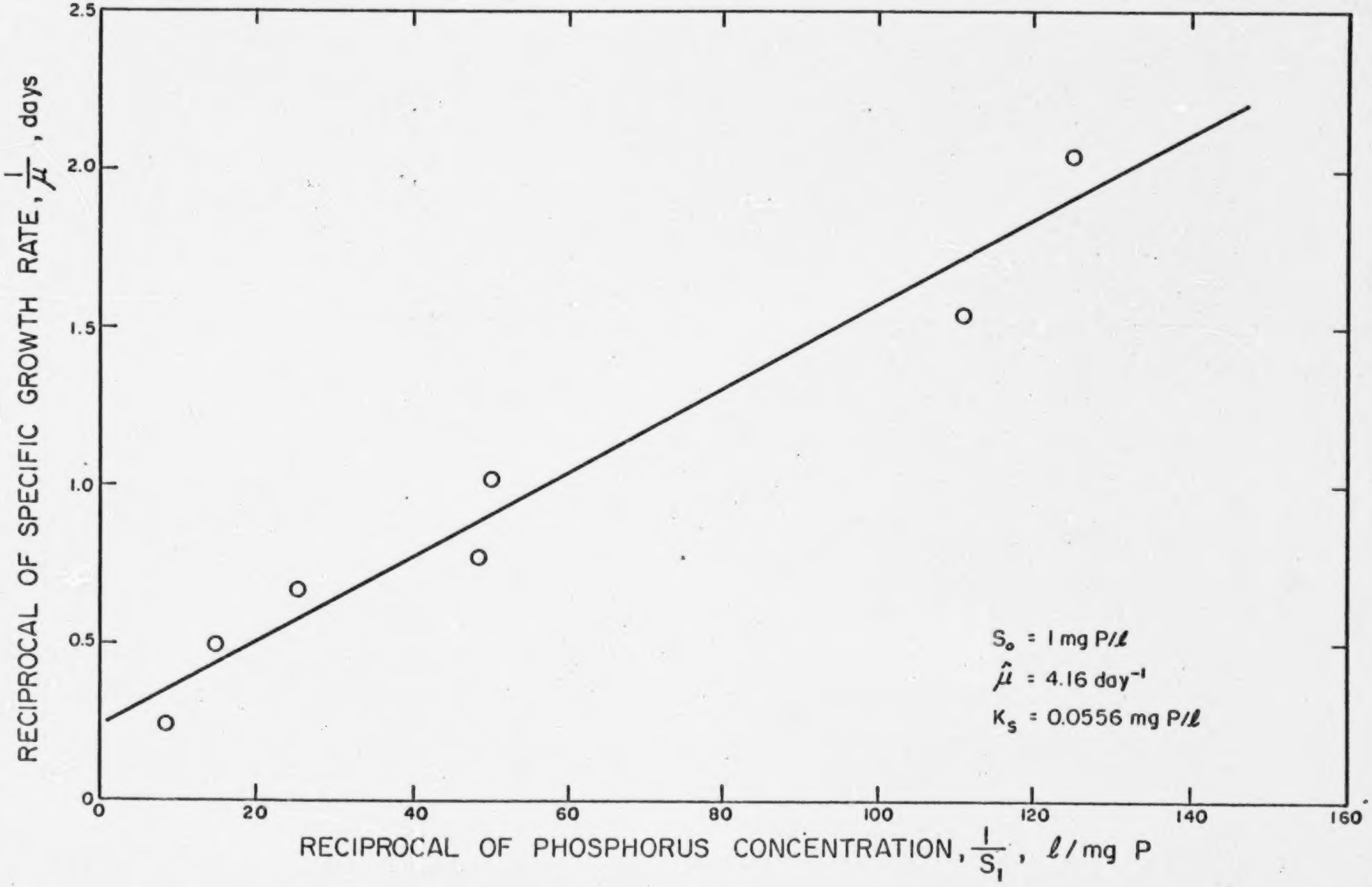

FIGURE 13. DETERMINATION OF THE MAXIMUM SPECIFIC GROWTH RATE, $\hat{\mu}$, AND SATURATION CONSTANT, K_s

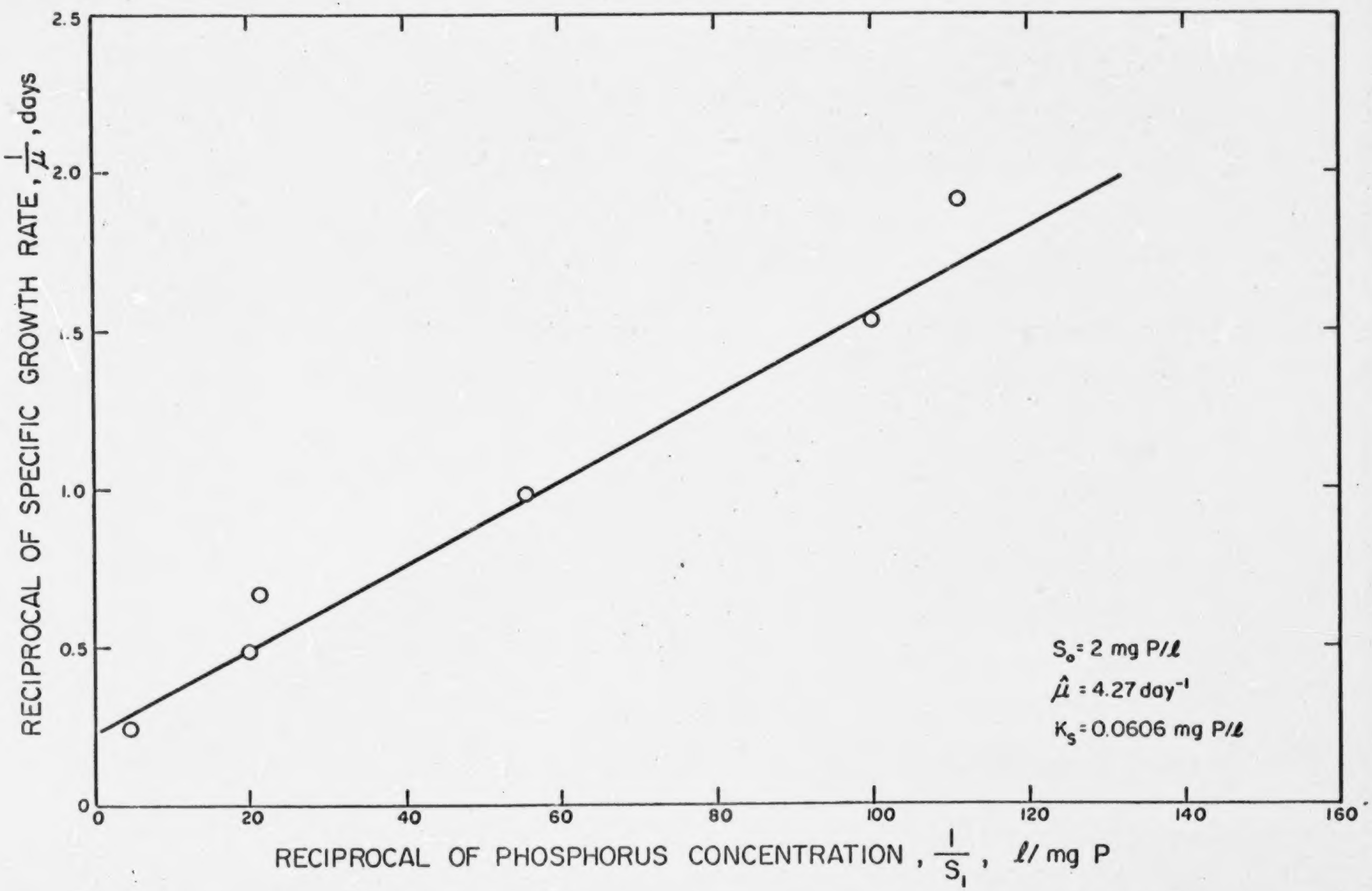

FIGURE 14. DETERMINATION OF THE MAXIMUM SPECIFIC GROWTH RATE, $\hat{\mu}$, AND SATURATION CONSTANT, K_s

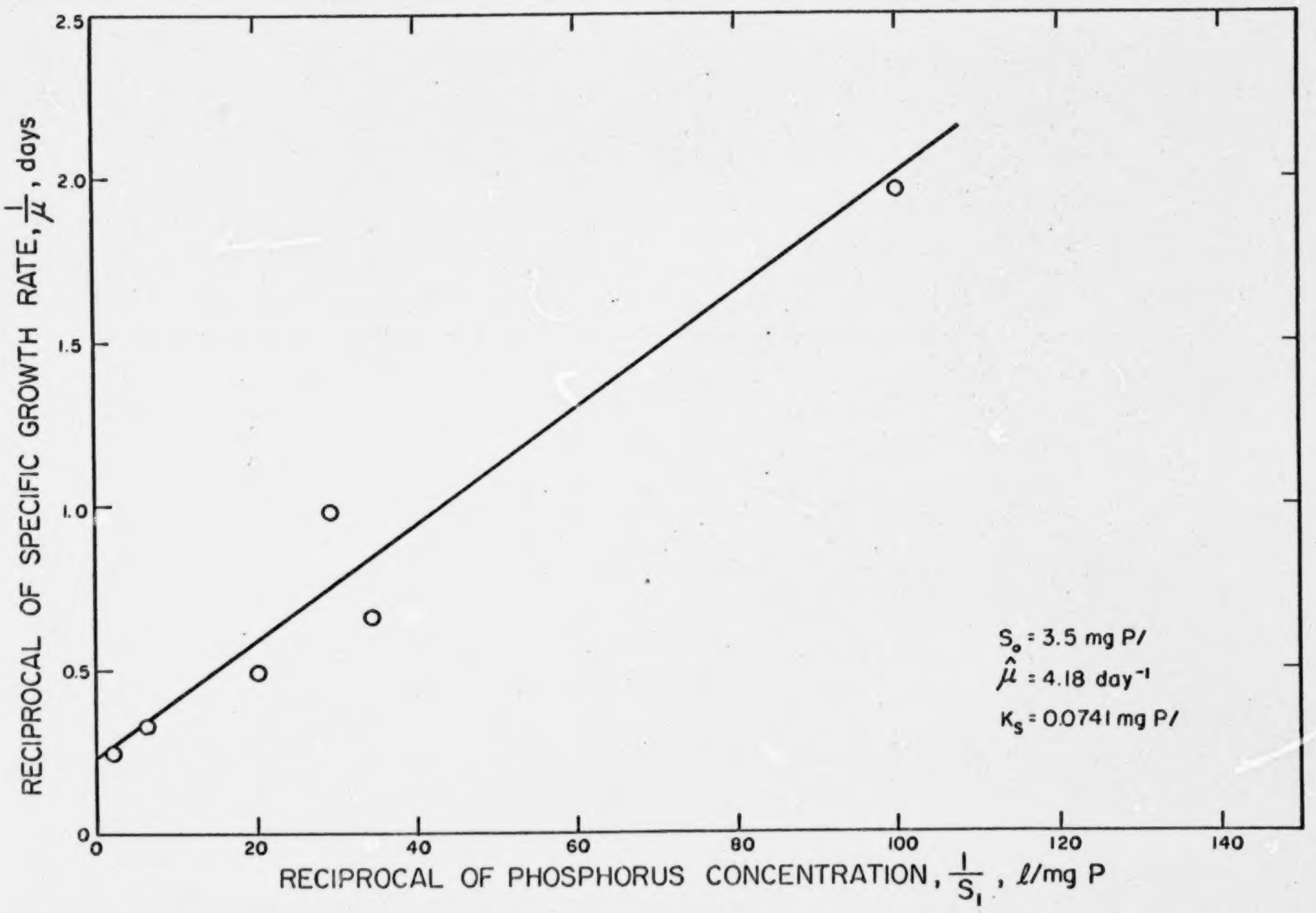

FIGURE 15. DETERMINATION OF THE MAXIMUM SPECIFIC GROWTH RATE, $\hat{\mu}$, AND SATURATION CONSTANT, K_s

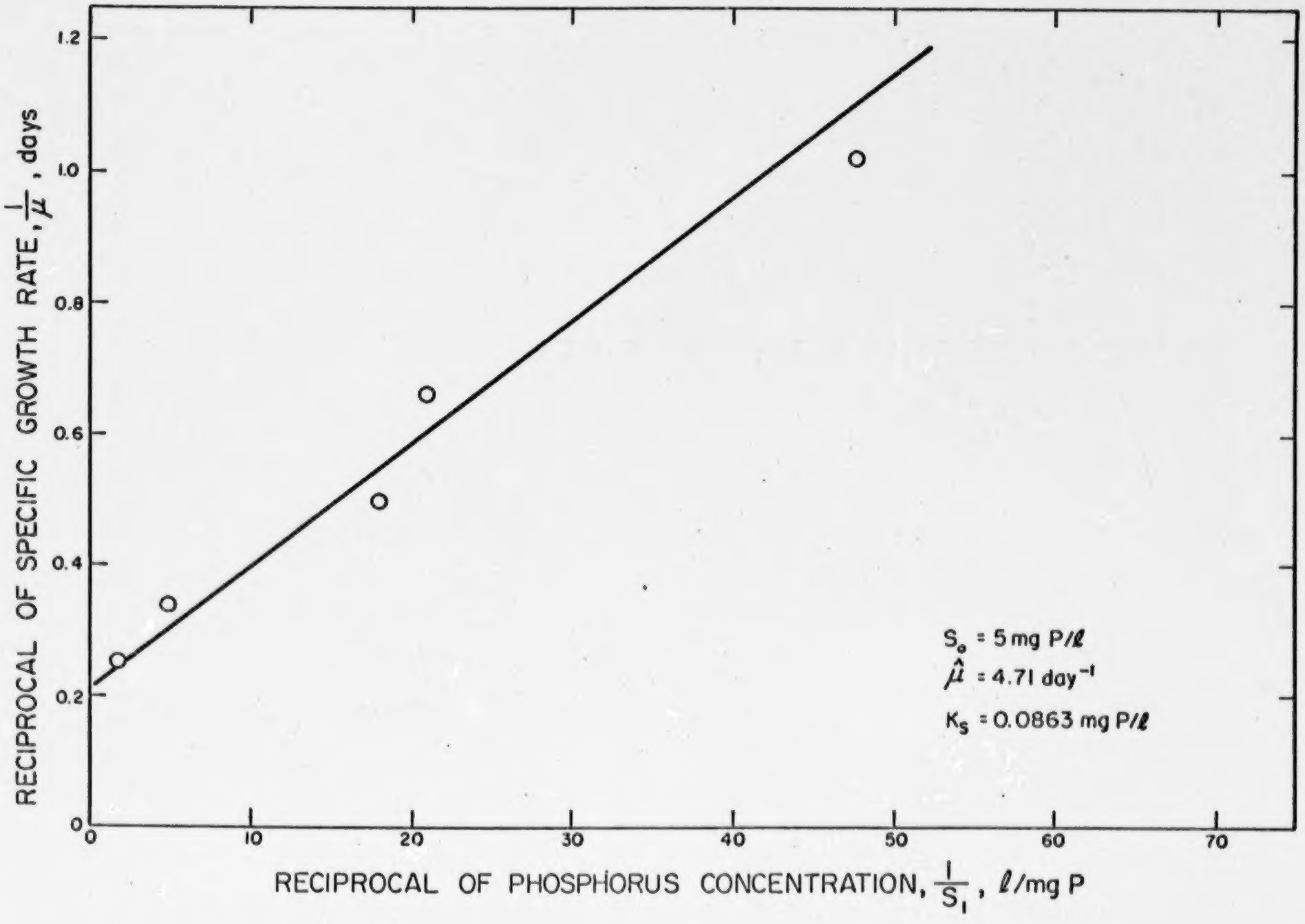

FIGURE 16. DETERMINATION OF THE MAXIMUM SPECIFIC GROWTH RATE, $\hat{\mu}$, AND SATURATION CONSTANT, K_s

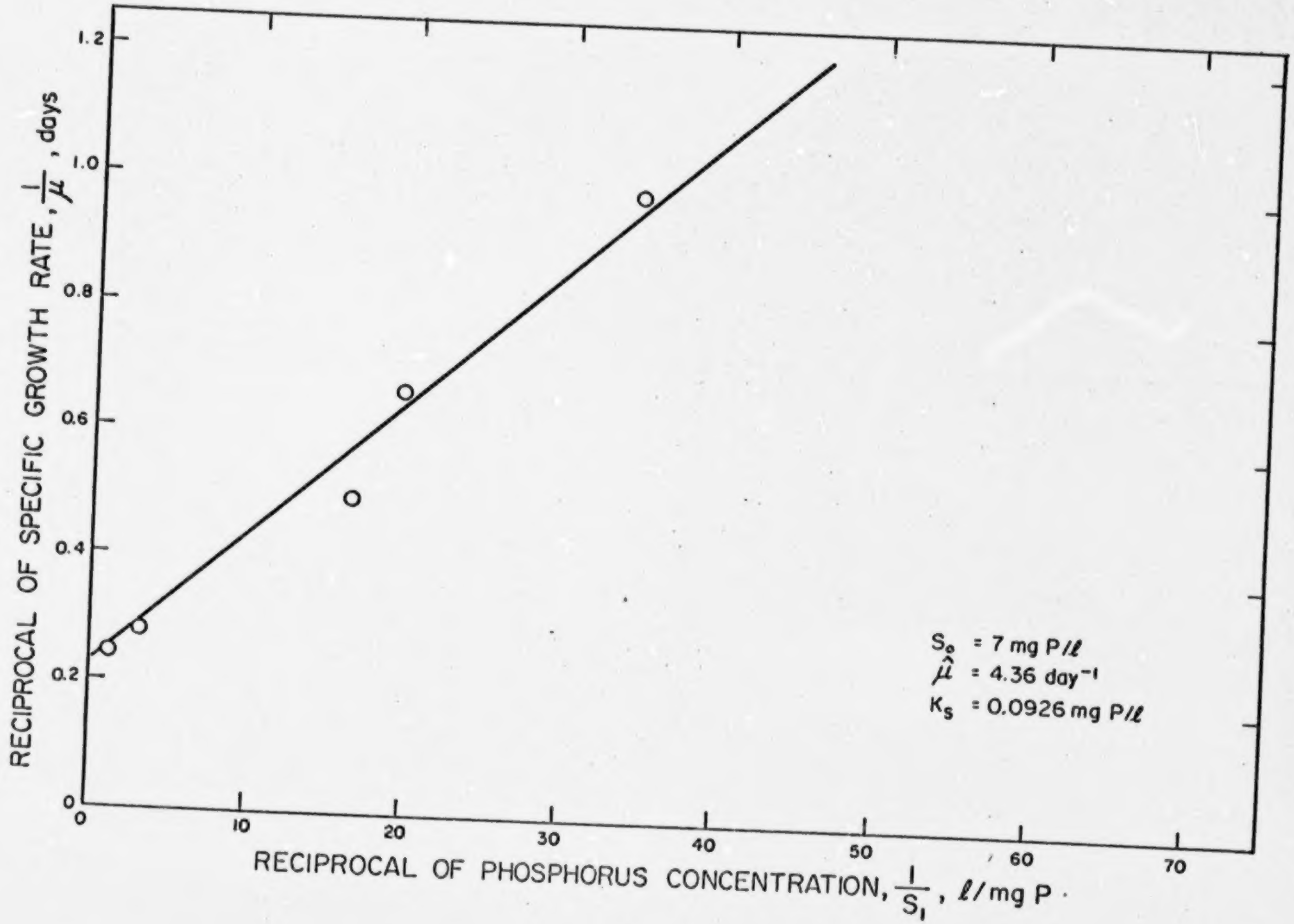

FIGURE 17. DETERMINATION OF THE MAXIMUM SPECIFIC GROWTH RATE, $\hat{\mu}$, AND SATURATION CONSTANT, K_s

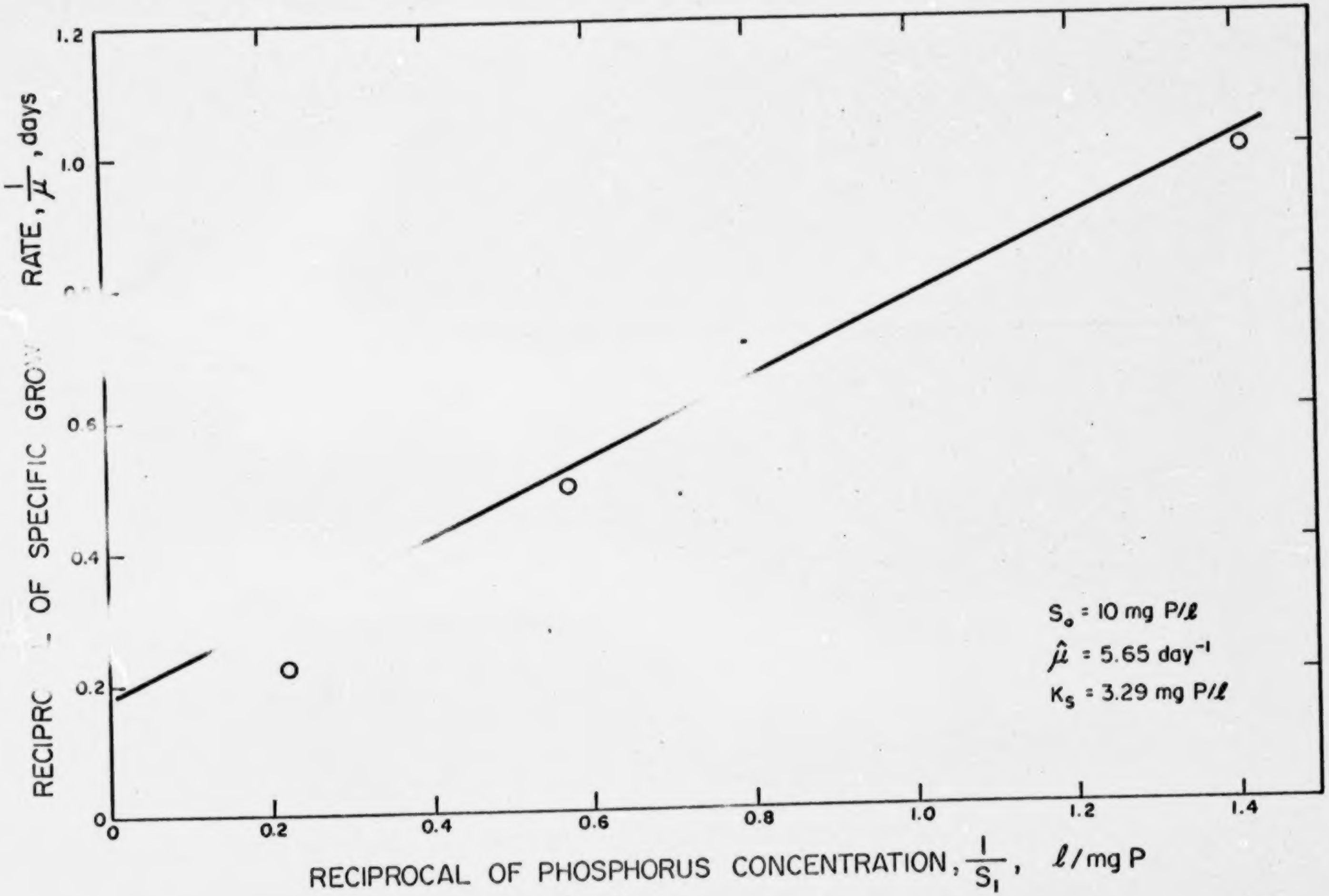

FIGURE 18. DETERMINATION OF THE MAXIMUM SPECIFIC GROWTH RATE, $\hat{\mu}$, AND SATURATION CONSTANT, K_s

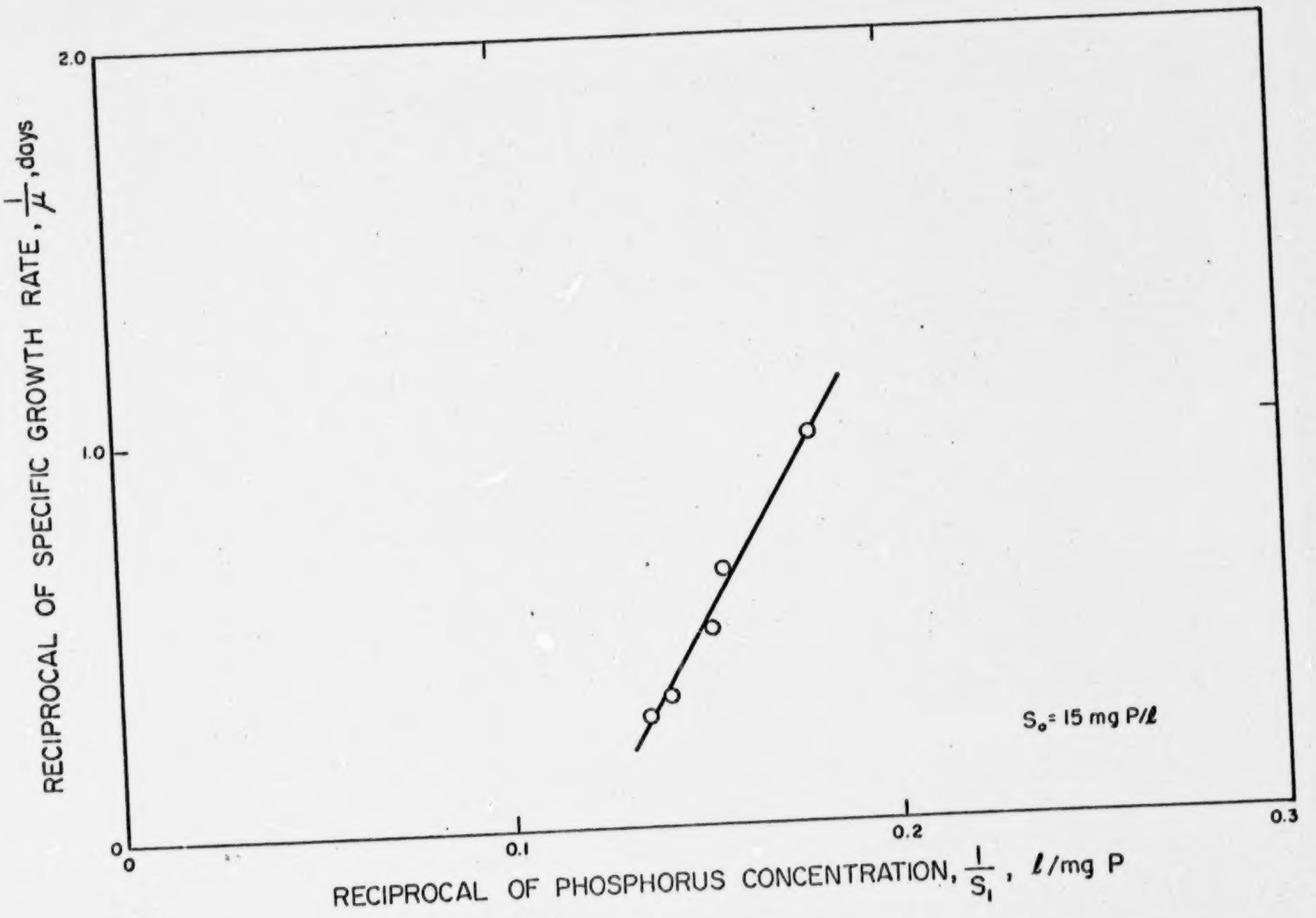

FIGURE 19. DETERMINATION OF THE MAXIMUM SPECIFIC GROWTH RATE, $\hat{\mu}$, AND SATURATION CONSTANT, K_s

well fitted by straight lines. The straight lines in the figures are the lines of best fit determined by the method of least squares. Table IX gives the values of the kinetic parameters, $\hat{u}$ and K_s, obtained from the equations of the best fit lines.

Examination of Table IX indicates that the values of $\hat{u}$ and K_s were not the same for all experiments. This is not unexpected since these values were determined from mathematically-derived equations. However, with the exception of the values from the experiments with high feed phosphorus levels, the values of $\hat{u}$ are close to each other. The value of K_s appears to increase with higher phosphorus concentration in the feed. It is believed, however, that the data is not sufficiently precise to establish that this variation is a real one. The data from the experiments using high phosphorus concentrations in the incoming medium produced results that were not consistent with those of the others.

An attempt was made to determine average values of $\hat{u}$ and K_s for those experiments which yielded comparable values of these parameters. This was done by calculating average phosphorus concentrations for each dilution rate and plotting the reciprocals as shown in Figure 20. From the equation of best fit, values of 4.19 day^{-1} and 0.0582 mg P/1 for $\hat{u}$ and K_s, respectively, were obtained. Figure 21 shows the theoretical curve of the Monod equation for these values of $\hat{u}$ and K_s together with the observed data.

The experimental data were also compared with other kinetic models. In Figure 22 are shown the plots to determine the fit of Teissier's equation to the data from two sets of experiments, using a method described by Schulze (195). Teissier's equation is as follows:

TABLE IX

VALUES OF $\hat{\mu}$ AND K_s - EXPERIMENTS WITH *C. pyrenoidosa* 71105

Expt. No.	Phosphorus in Feed, S_o mg P/l	Maximum Specific Growth Rate, $\hat{\mu}$ day^{-1}	Saturation Constant, K_s mg P/l
A-I	0.5	4.21	0.0544
A-II	1.0	4.16	0.0556
A-III	2.0	4.27	0.0606
A-IV	3.5	4.18	0.0741
A-V	5.0	4.71	0.0863
A-VI	7.0	4.36	0.0926
A-VII	10.0	5.65	3.29
A-VIII	15.0	--	--

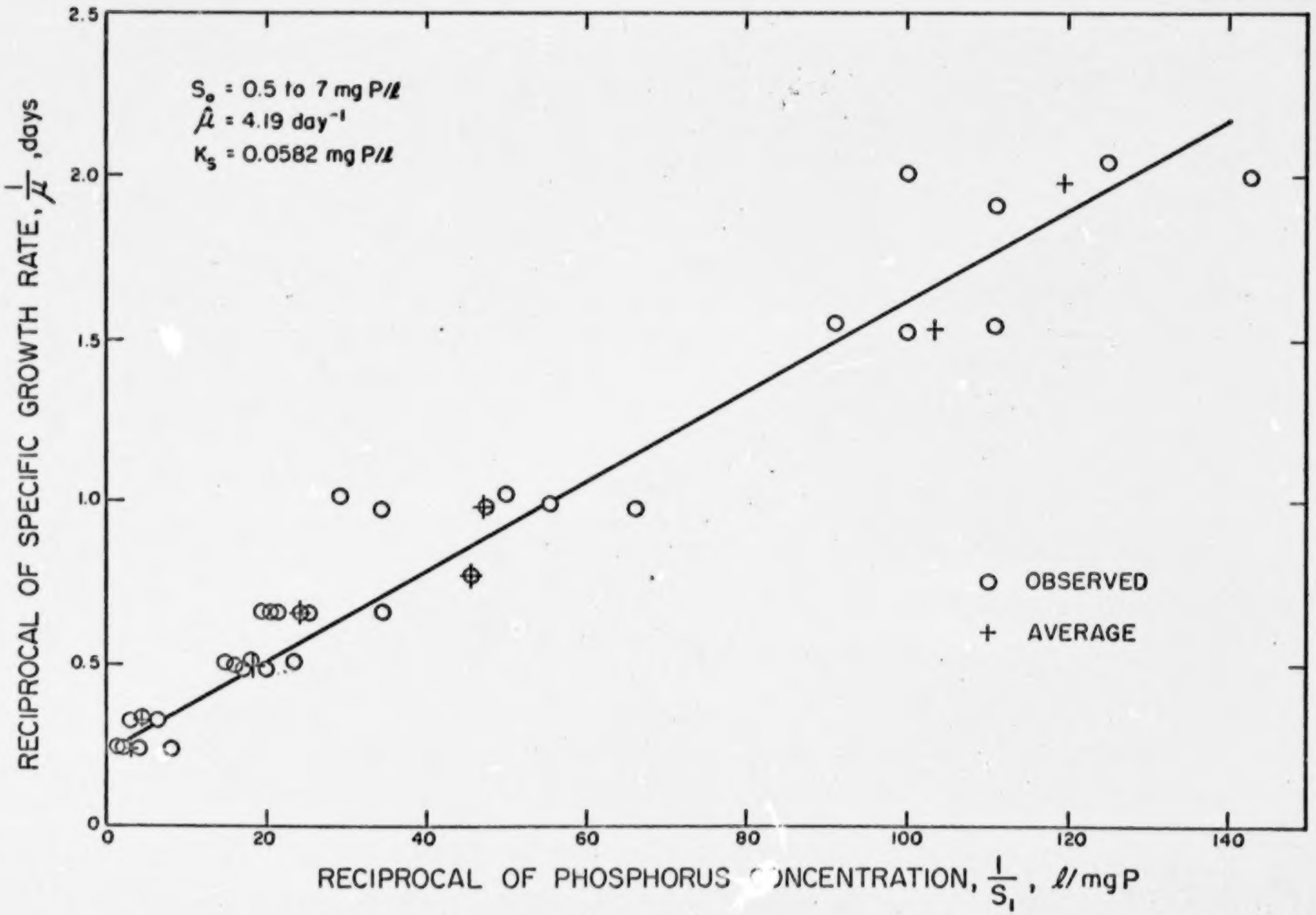

FIGURE 20. DETERMINATION OF THE MAXIMUM SPECIFIC GROWTH RATE, $\hat{\mu}$, AND SATURATION CONSTANT, K_s

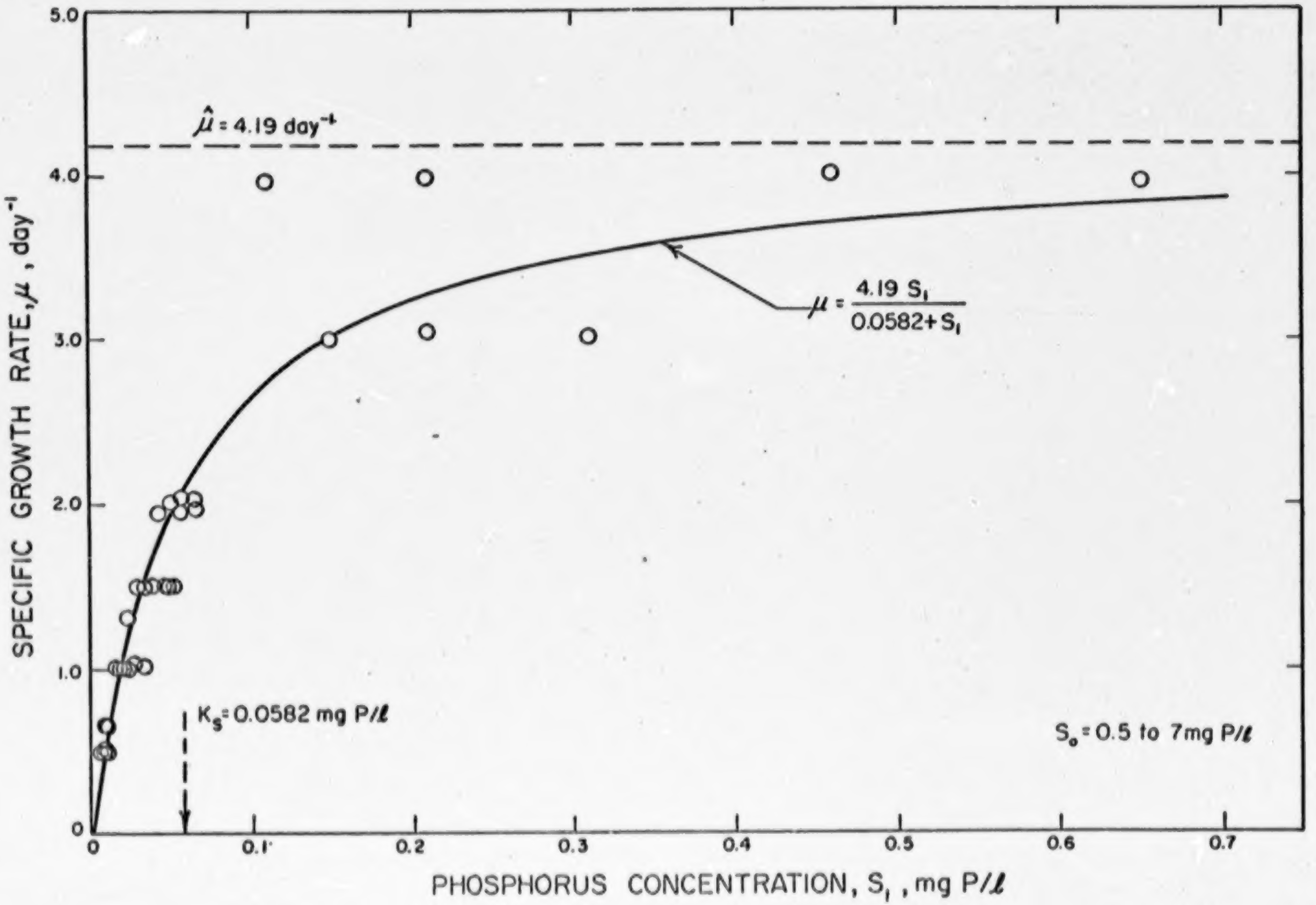

FIGURE 21. GROWTH RATE AS A FUNCTION OF PHOSPHORUS CONCENTRATION (C. Pyrenoidosa 71105; T = 39 ± 0.5 °C; pH = 7.5 ± 0.1)

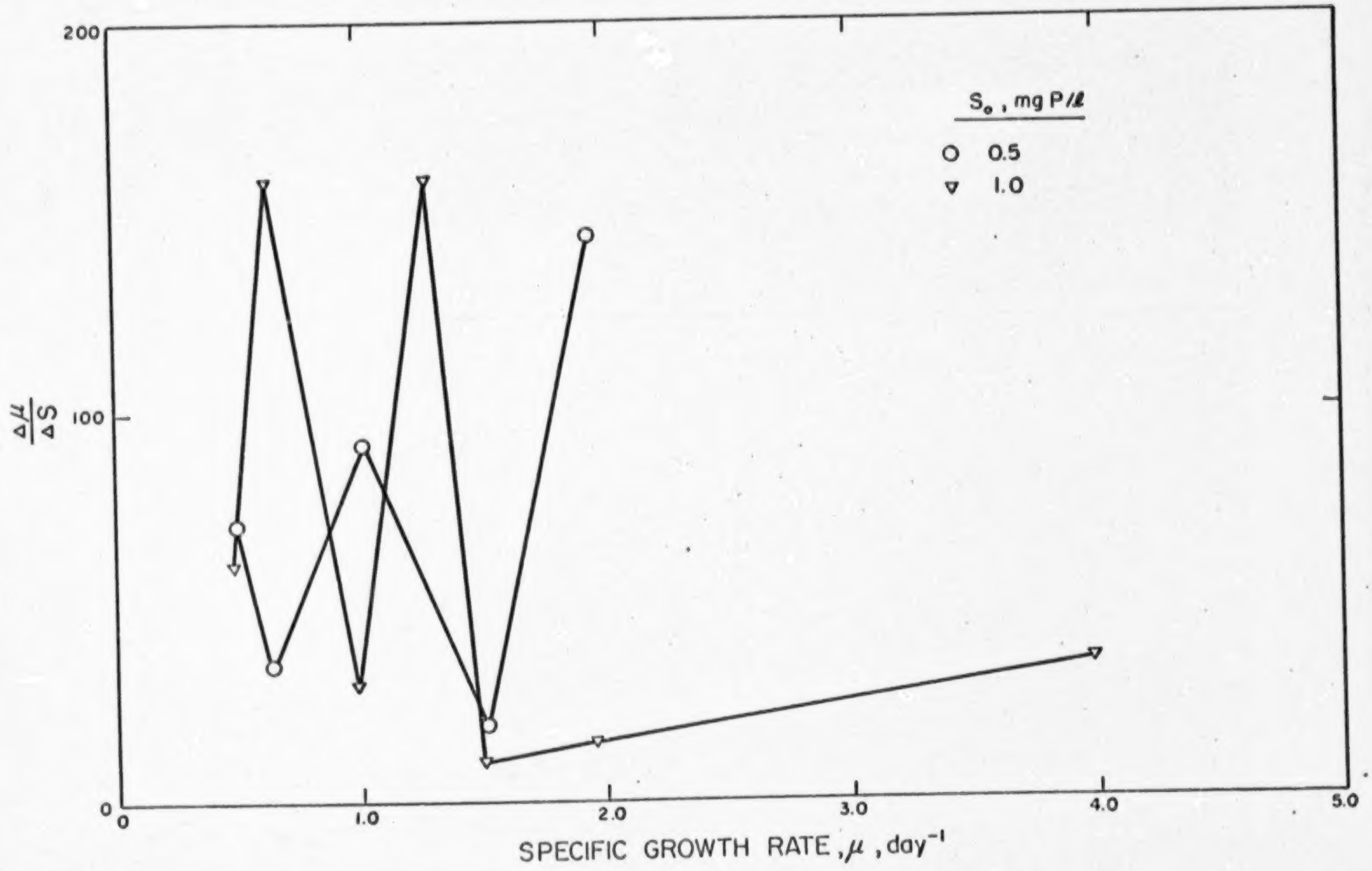

FIGURE 22. PLOT OF $\Delta\mu/\Delta S$ VS μ FOR TESTING FIT OF TEISSIER'S KINETIC MODEL TO EXPERIMENTAL DATA

$$u = \hat{u}(1 - e^{-S/K_s}) \qquad (7)$$

This equation can also be written as

$$\frac{du}{dS} = \frac{\hat{u}}{K_s} - \frac{u}{K_s}$$

or

$$\frac{\Delta u}{\Delta S} = \frac{\hat{u}}{K_s} - \frac{u}{K_s} \qquad (44)$$

Equation 44 is an equation of a straight line in which the slope is $1/K_s$, the y-intercept is $\hat{u}/K_s$, and the x-intercept is $\hat{u}$.

From Figure 22 it can be seen that the Teissier model did not give a good fit to the experimental data. Comparison of the data from the other experiments was, therefore, no longer made.

The suitability of Teissier's model can also be evaluated in another way. Teissier's equation can also be written in the following form:

$$\log_e \left(\frac{\hat{u} - u}{\hat{u}}\right) = -\frac{S}{K_s} \qquad (45)$$

or

$$\log_{10} \left(\frac{\hat{u}}{\hat{u} - u}\right) = \frac{S}{2.3K_s} \qquad (46)$$

A plot of $\log_{10} \left(\frac{\hat{\mu}}{\hat{\mu} - \mu}\right)$ against S is a straight line passing through the origin with a positive slope of $1/2.3K_s$. The above form of Teissier's equation does not permit direct determination of $\hat{\mu}$ from the data but can be used to determine K_s once the value of $\hat{u}$ has been estimated or established from batch cultures or other methods.

Using the values of $\hat{\mu}$ given in Table IX, plots of the same data used in Figure 22 according to equation are shown in Figures 23 and 24.

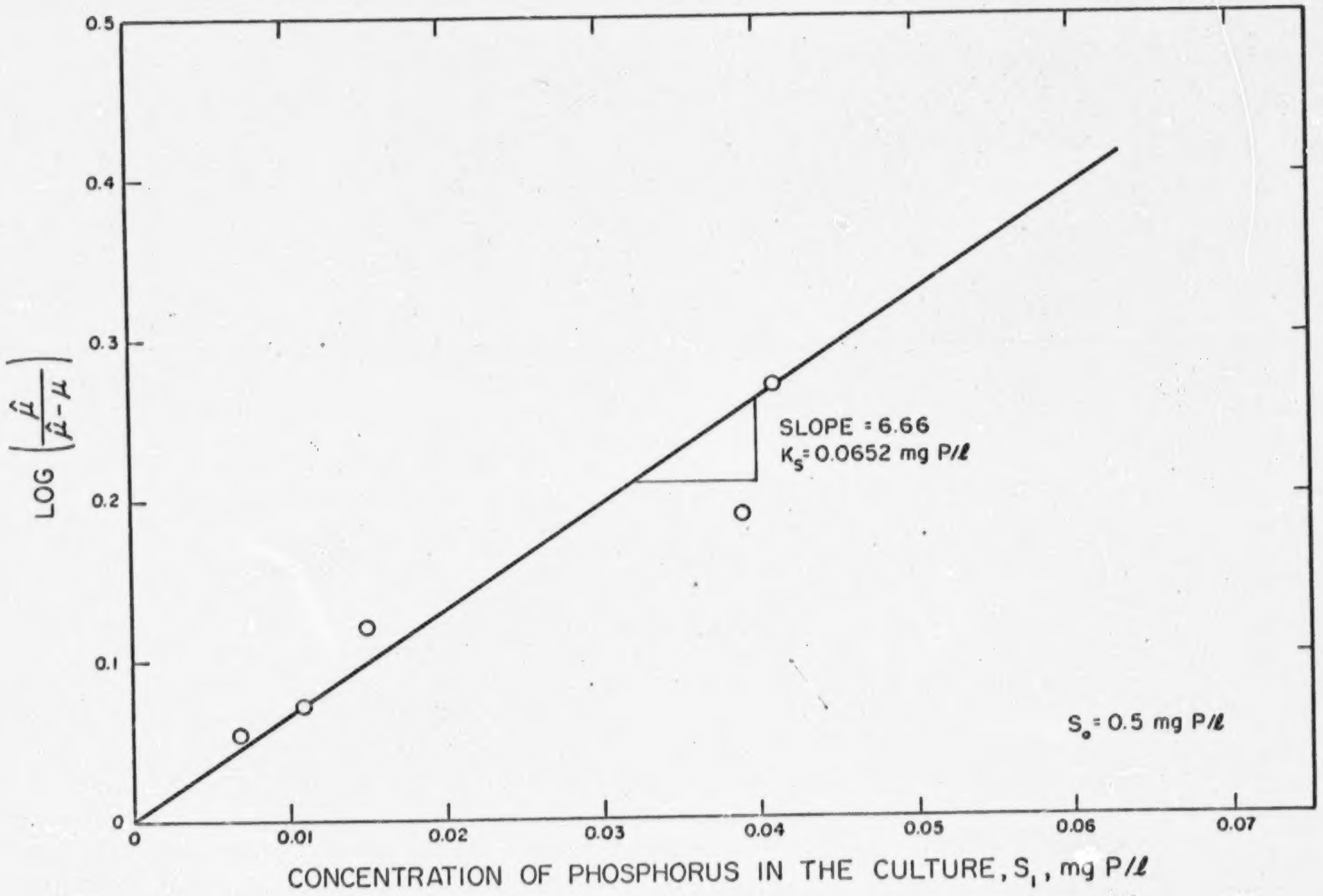

FIGURE 23. PLOT OF $\mathrm{LOG}\left(\frac{\hat{\mu}}{\hat{\mu}-\mu}\right)$ VS S_1 FOR TESTING FIT OF TEISSIER'S KINETIC MODEL TO EXPERIMENTAL DATA

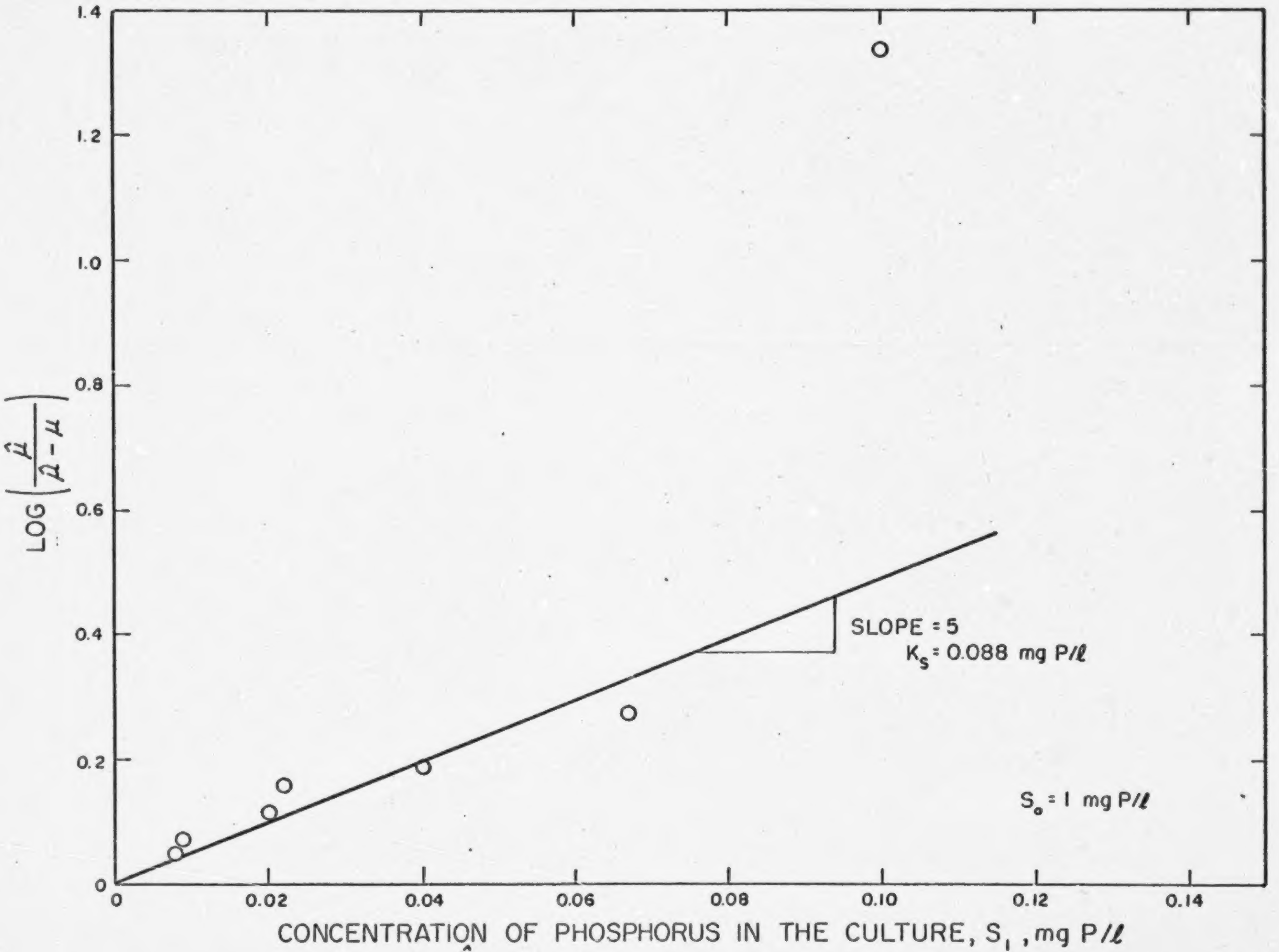

FIGURE 24. PLOT OF LOG $\left(\frac{\hat{\mu}}{\hat{\mu}-\mu}\right)$ VS S_1 FOR TESTING FIT OF TEISSIER'S KINETIC MODEL TO EXPERIMENTAL DATA

As can be seen, this method gave a better fit of Teissier's equation to the data than the other method described above. Comparison of these plots with the corresponding double reciprocal plots of the data, however, indicates that the Monod equation provides a more satisfactory fit than Teissier's equation. The lines shown in Figures 23 and 24 were drawn by eye. Values of K_s obtained from these lines were higher than those obtained from the double reciprocal plots.

The suitability of Moser's kinetic model was also tested, using as estimates of $\hat{\mu}$ and K_s the values given in Table IX. Moser's equation is

$$\mu = \hat{\mu} \left(\frac{1}{1 + K_s S^{-n}} \right) \qquad (5)$$

where n is a constant. Figures 25 and 26 show the theoretical curves of Moser's equation compares with the experimental data. It will be noted that the curves for a value of n of 1 gave the best fit to the data. With this value of n, Moser's equation is the same as that of Monod.

ALGAL CONCENTRATION

The steady-state cell concentration is one of the parameters characterizing continuous cultures. The steady state cell concentration adjusts to the supply of the limiting nutrient to the continuous culture, which in turn depends on the flow rate of the feed or the dilution rate. Figure 27 shows the relationship between the steady state concentration of algae observed in this study and the dilution rate. The values of the algal concentration are listed in column 6 of Table VIII.

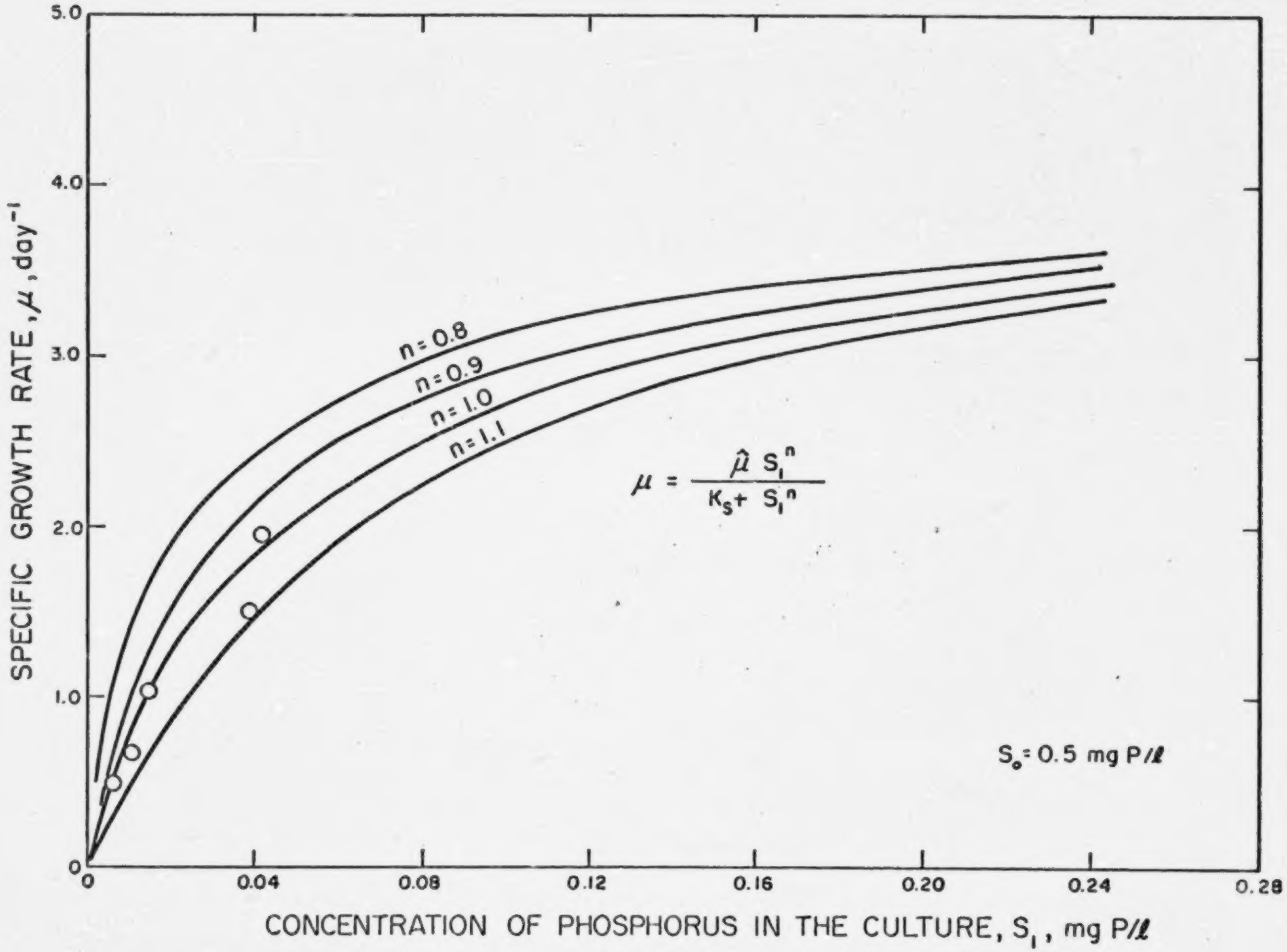

FIGURE 25. COMPARISON OF EXPERIMENTAL DATA TO THEORETICAL CURVES ACCORDING TO MOSER'S KINETIC MODEL

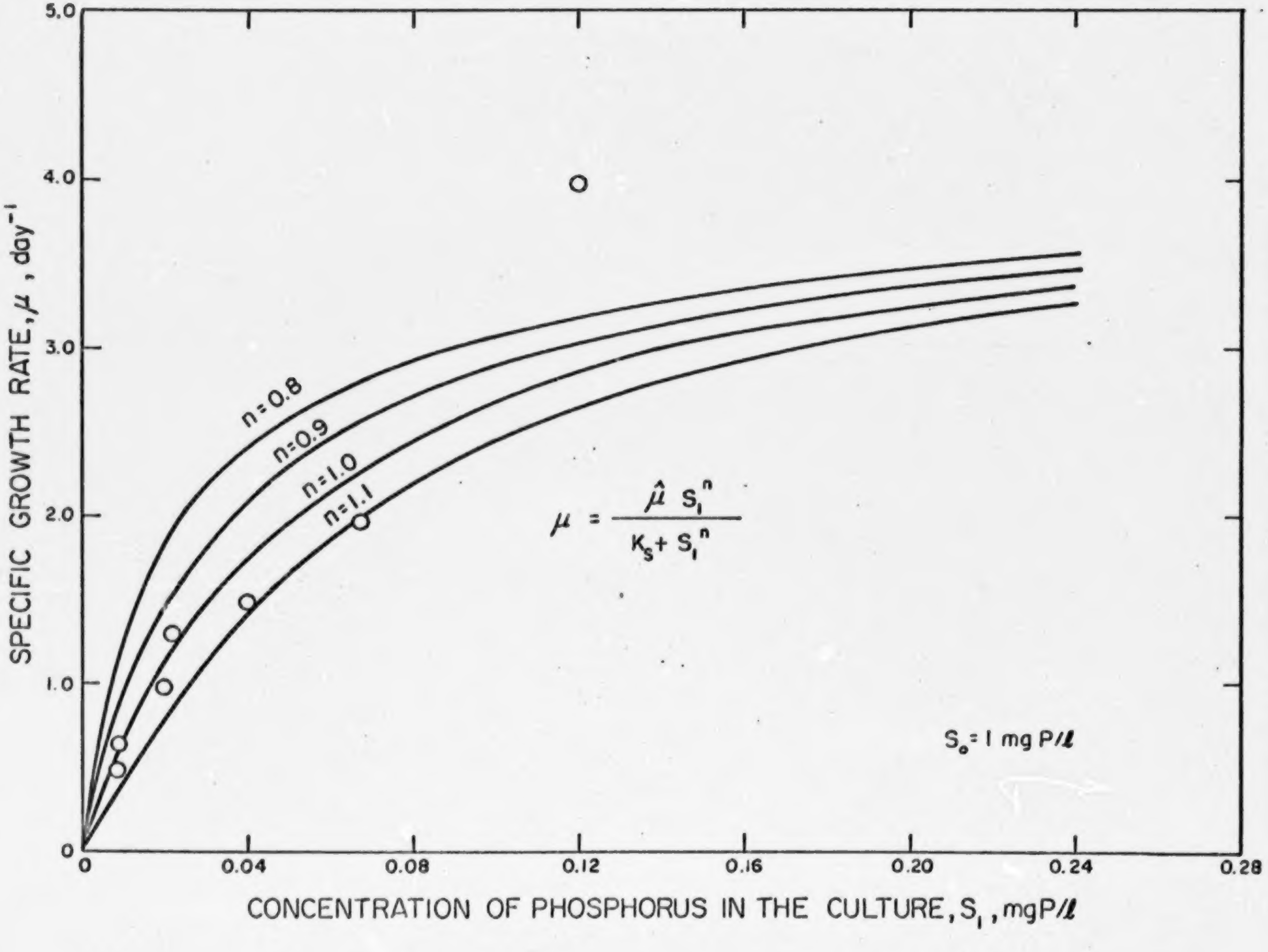

FIGURE 26. COMPARISON OF EXPERIMENTAL DATA TO THEORETICAL CURVES ACCORDING TO MOSER'S KINETIC MODEL

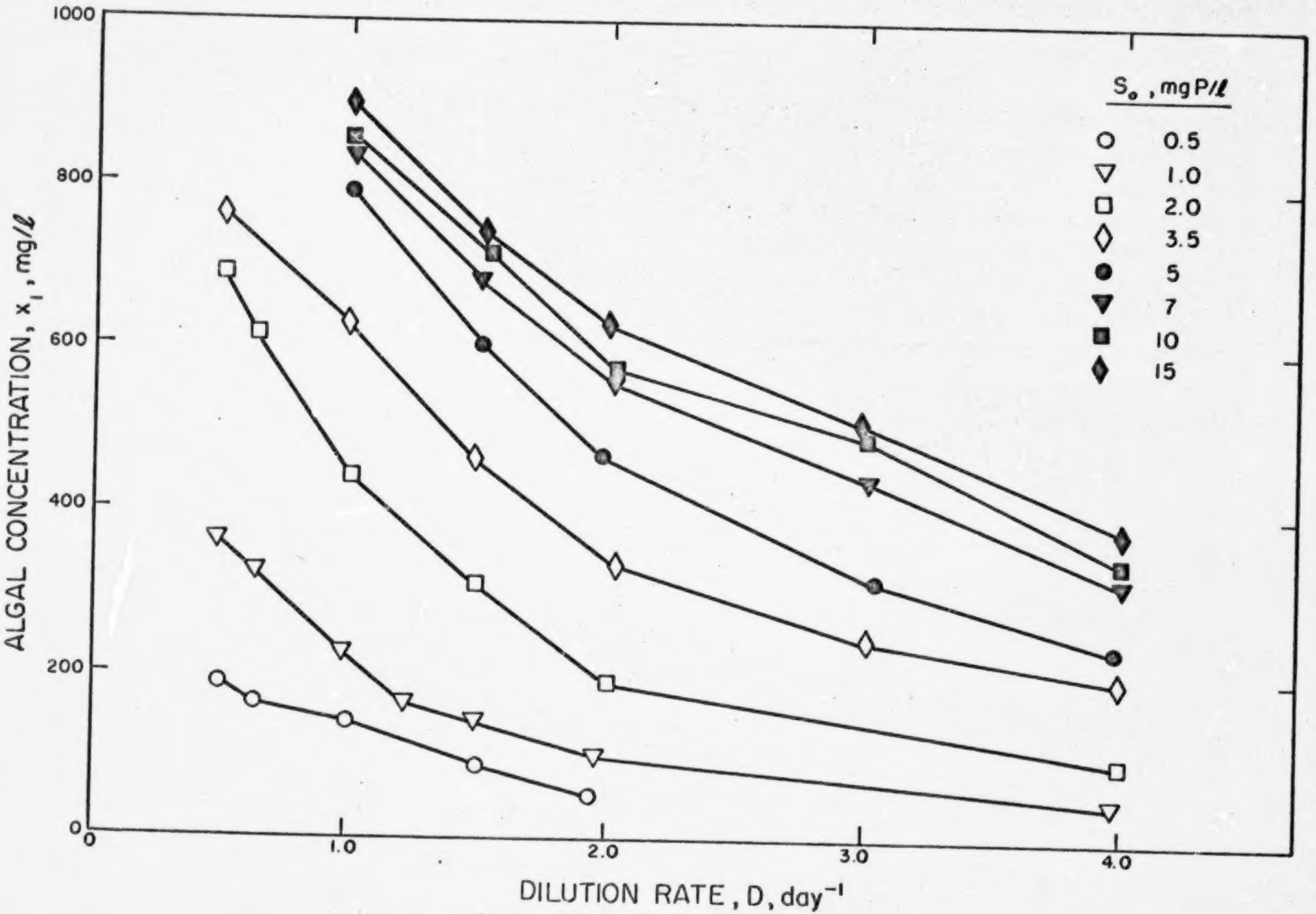

FIGURE 27. ALGAL CONCENTRATION AS A FUNCTION OF DILUTION RATE

Figure 27 shows that the algal concentration declines as the dilution rate increases. This apparently is a characteristic of algal cultures. Other workers (191, 207) have observed a varying equilibrium cell concentration with dilution rate in studies employing chemostatic continuous cultures. The data in Figure 27 indicate that for certain ranges of dilution rate, the steady state concentration of algae is approximately inversely proportional to the dilution rate. As is to be expected, the algal concentrations were greater for higher levels of phosphorus in the feed.

Algal concentrations plotted as a function of the detention period are shown in Figure 28. An approximate linear relationship can be seen to exist between the two but the curves tended to level off as the detention time increased indicating that the algal concentration approaches a maximum value.

The effect of the phosphorus level, S_0, in the incoming feed on the equilibrium algal concentration is also shown in Figure 29. It is evident that the algal concentration is also proportional to the phosphorus in the incoming medium up to certain levels of phosphorus.

CELL YIELD AND PHOSPHORUS CONSUMPTION

Values of the cell yield with respect to phosphorus were obtained by dividing the steady state algal concentration by the difference between the phosphorus concentration in the incoming medium and that in the culture, or

$$Y = \frac{x_1}{S_0 - S_1} \qquad (47)$$

where Y = cell yield, mg cells/mg phosphorus consumed. The results are given in column 9 of Table VIII.

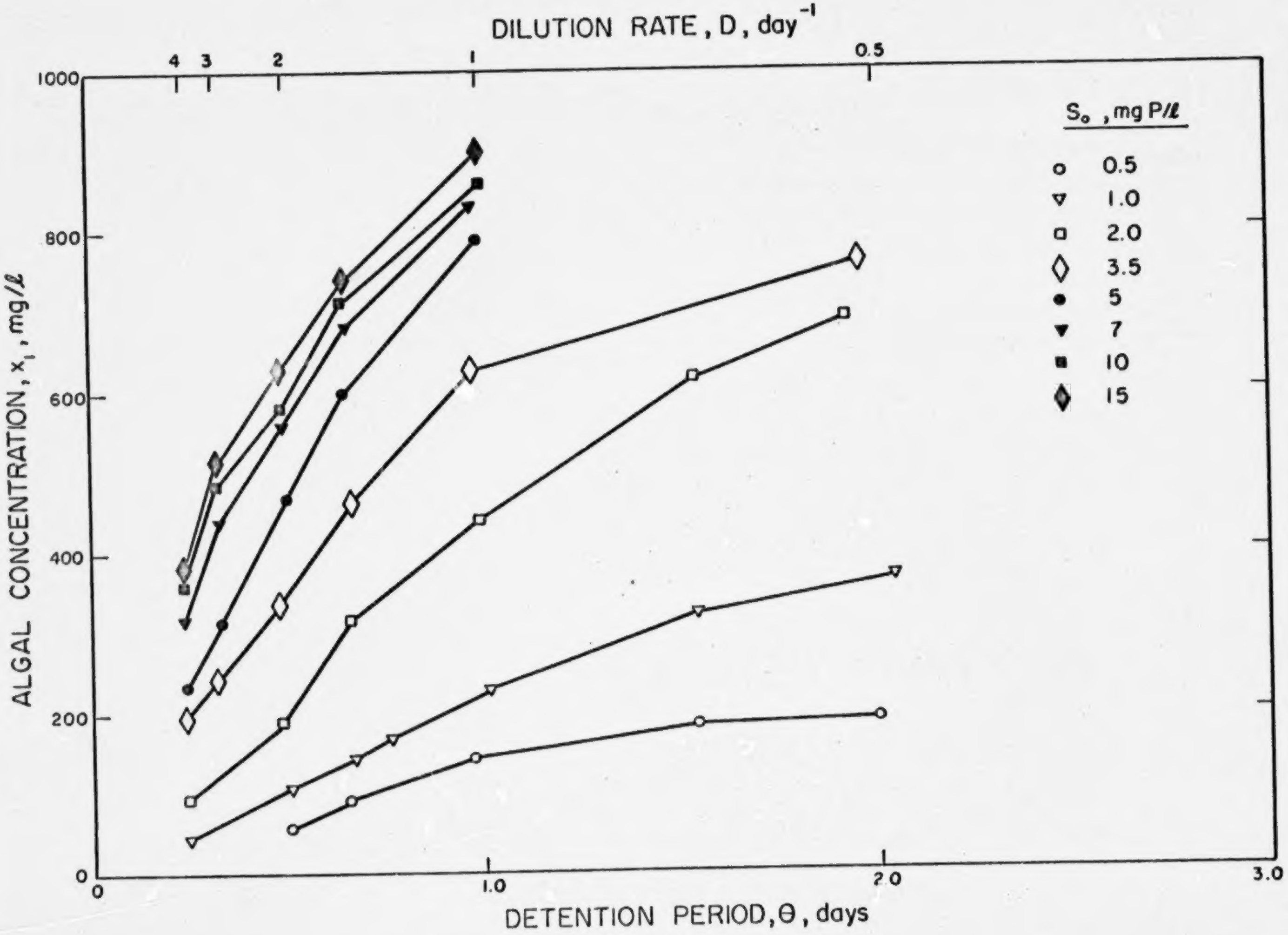

FIGURE 28. ALGAL CONCENTRATION AS A FUNCTION OF DETENTION PERIOD

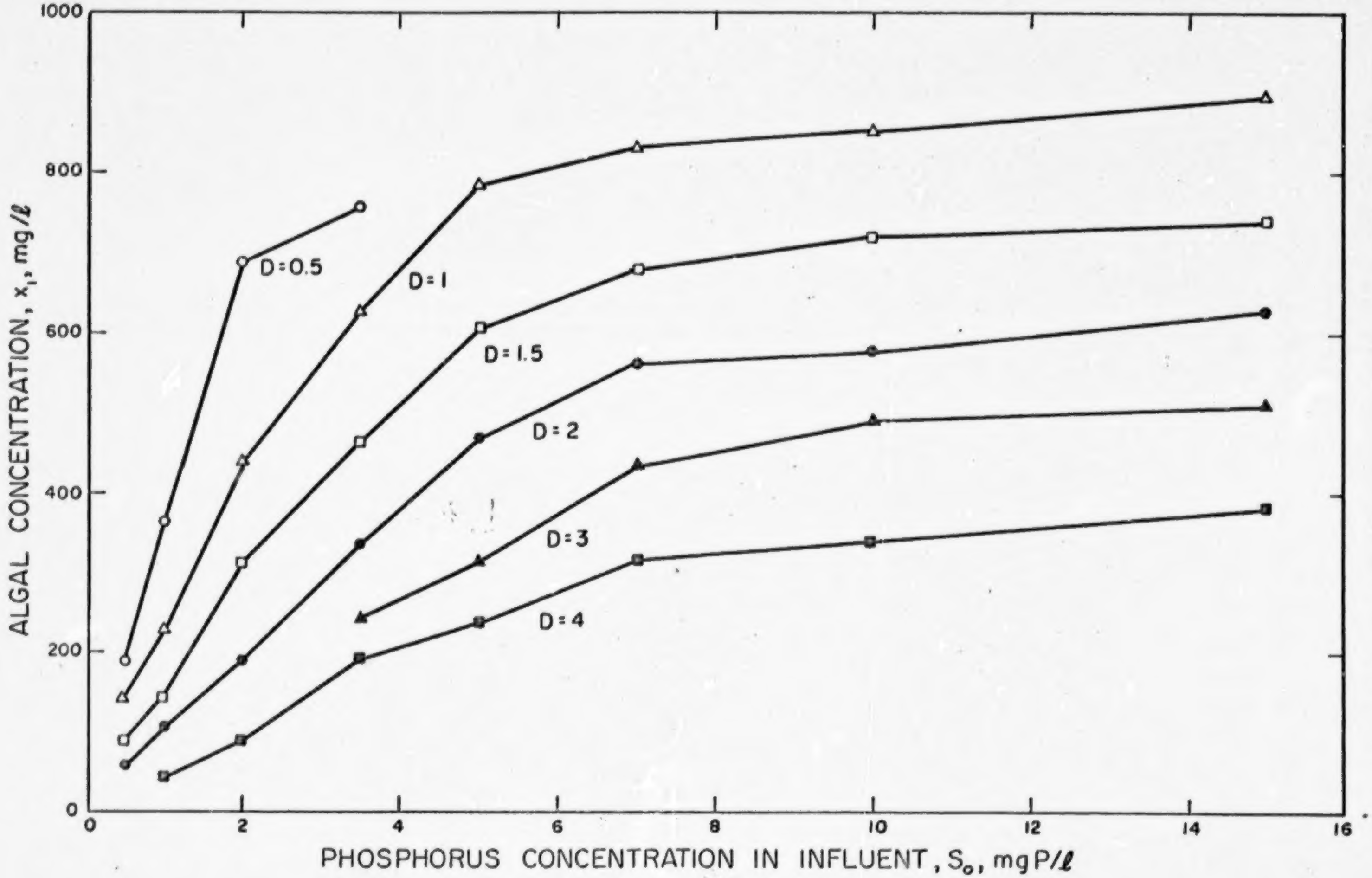

FIGURE 29. RELATIONSHIP BETWEEN ALGAL CONCENTRATION AND PHOSPHORUS CONCENTRATION IN INFLUENT

From Table VIII it can be seen that the yield was not constant but varied with the dilution rate. Evidently, the finding or common assumption of a constant yield with respect to carbon and other nutrients does not hold true for phosphorus. Plots of the cell yield as a function of dilution rate are shown in Figures 30 and 31. The yield followed the trend of the algal concentration, declining as the dilution rate was increased. This also means that the consumption of phosphorus per unit mass of cells was greater at the higher dilution rates. Viewed in another way, the algae were less efficient in the utilization of phosphorus in biomass synthesis at the higher dilution rates. With a phosphorus level in the feed of 0.5 to 3.5 mg P/1 and a dilution rate of 0.5 per day, the yield ranged from 374 to 219 mg cells per mg phosphorus consumed. This range corresponds to a phosphorus content of the cell mass of 0.27 to 0.46 per cent. The yield dropped to 124 to 97 mg cells per mg phosphorus consumed at a dilution rate of 2 per day, corresponding to a phosphorus content of the cell mass of 0.81 to 1.03 per cent. With further increase in the dilution rate to 4 per day, the yield decreased to 64 to 53 mg cells per mg phosphorus consumed. The phosphorus content of the cell mass for these values of the yield are 1.56 to 1.89 per cent. For any dilution rate, the higher yield was generally associated with the smaller phosphorus level in the feed.

From Figures 30 and 31, it appears that for ranges of dilution rates below and above 2 per day, the relation between the yield and dilution rate can be described by a linear relationship. In the lower range, the relationship also depends on the phosphorus concentration in the feed. For dilution rates above 2 per day, the

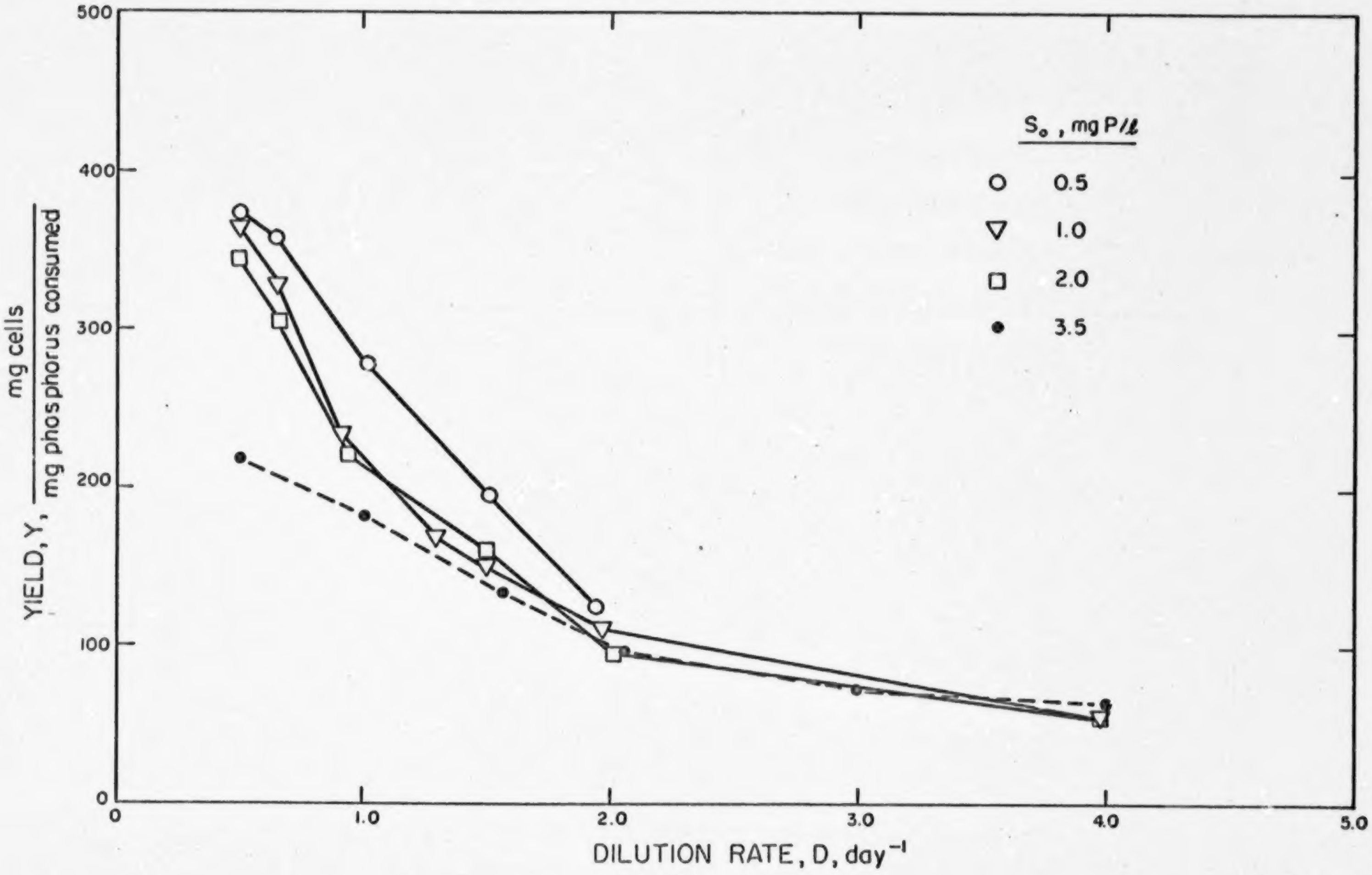

FIGURE 30. RELATIONSHIP BETWEEN YIELD AND DILUTION RATE

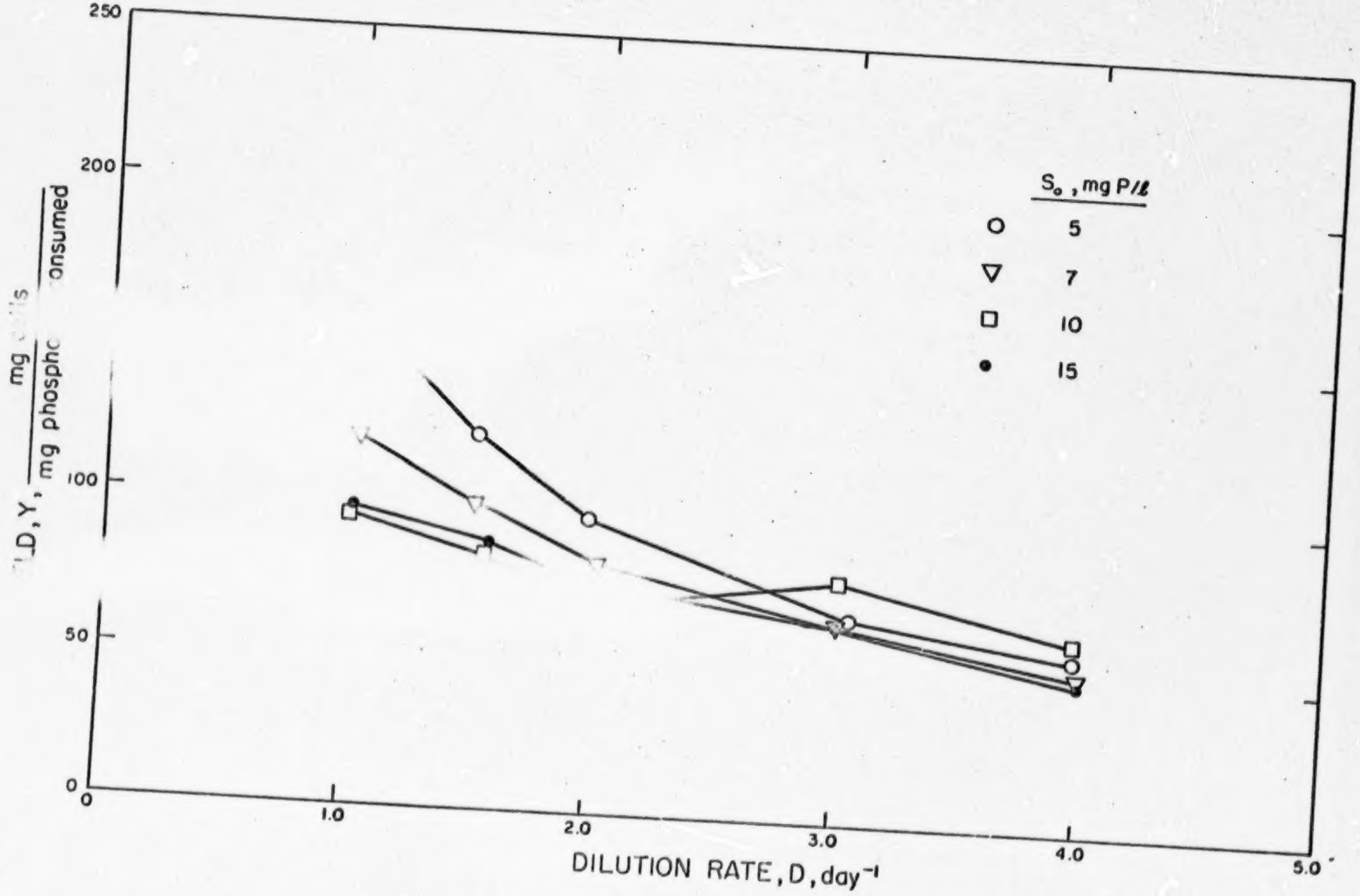

FIGURE 31. RELATIONSHIP BETWEEN YIELD AND DILUTION RATE

variation in yield with the dilution rate and also with the feed phosphorus is small so that an average yield factor can probably be assumed.

The relationship between yield and detention period are illustrated in Figures 32 and 33. An approximate proportionality between the yield and detention period is exhibited up to certain values of the detention period.

The effect of the phosphorus level in the incoming medium on yield is shown in Figure 34. At the small dilution rates, the yield decreases rapidly with increase in the phosphorus level, indicating that the algae took up more phosphorus per unit mass of cells with greater availability of the nutrient. The effect of the feed phosphorus level became less significant, however, at dilution rates of 2 per day and higher.

ALGAL PRODUCTION

Algal production rates are important in the use of algal cultures for nutrient removal since they determine the amount of algal material to be handled and disposed. The cell production rate of a continuous flow system is the product of the effluent flow rate and the concentration of organism in the effluent. Since, in chemostatic cultures the influent flow rate is equal to the effluent flow rate and the cell concentration in the effluent equal to that of the culture, the production rate can be expressed as

$$P = Fx_1 \tag{48}$$

where P = cell production rate, mass/time. The production rate

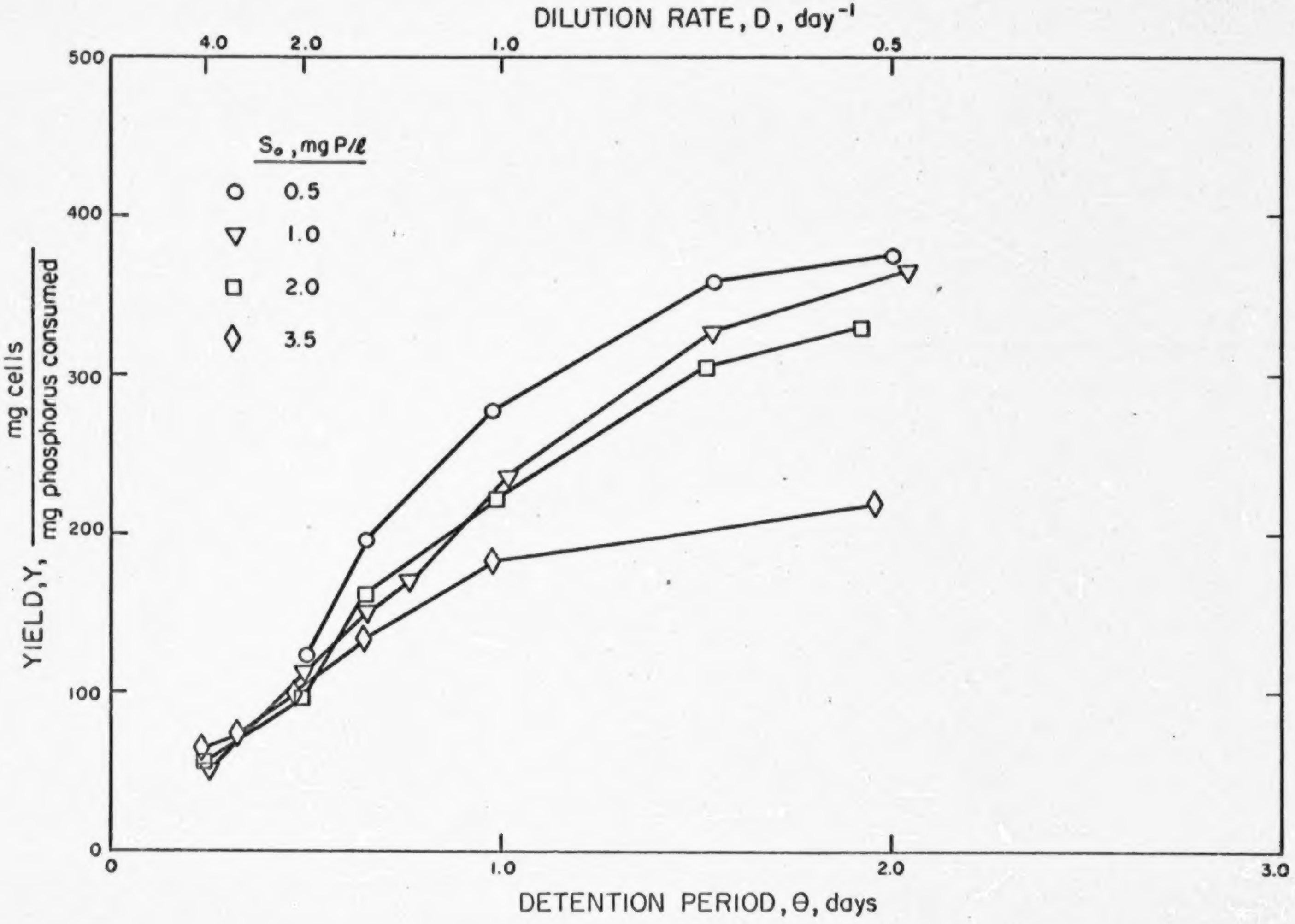

FIGURE 32. RELATIONSHIP BETWEEN YIELD AND DETENTION PERIOD

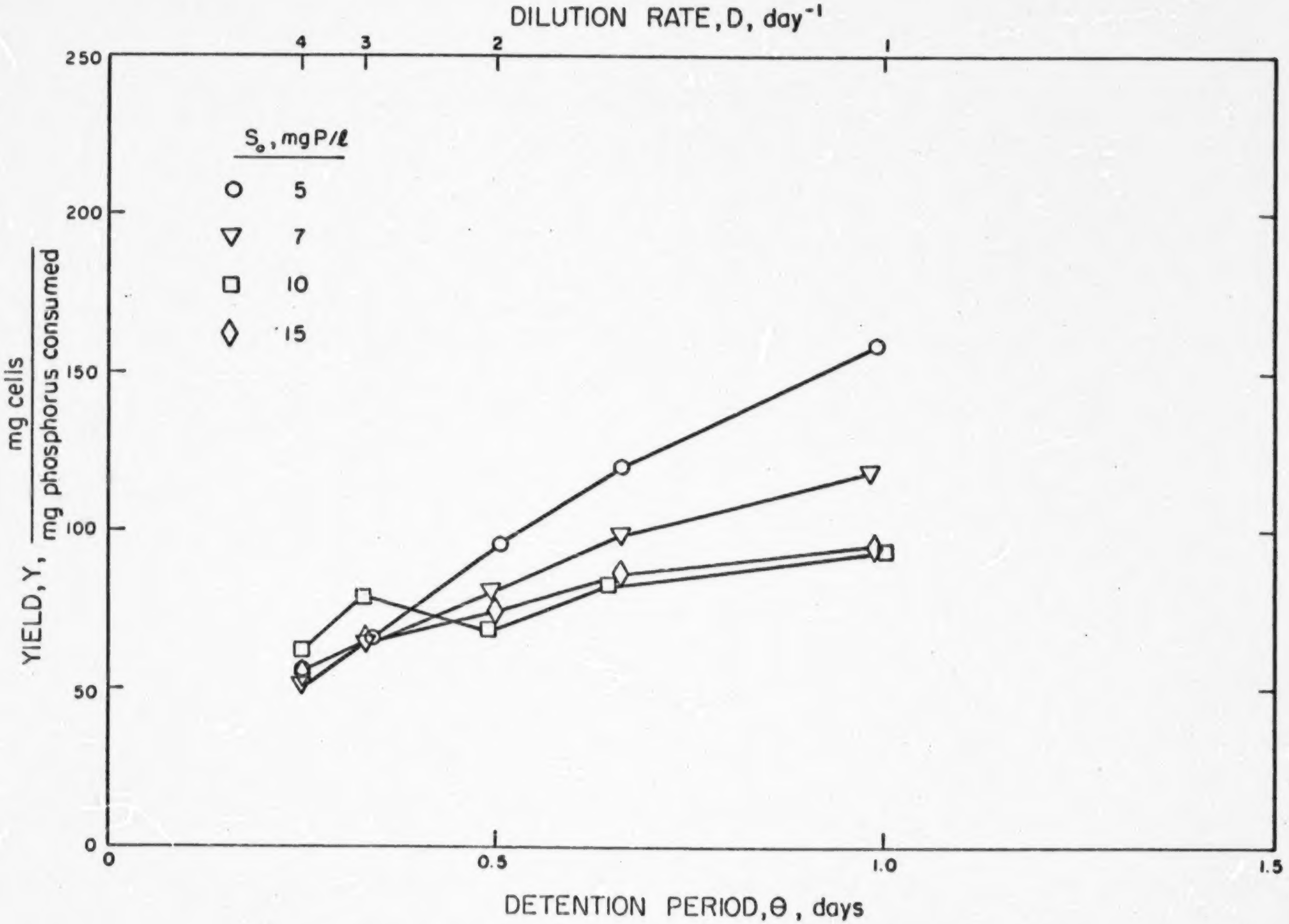

FIGURE 33. RELATIONSHIP BETWEEN YIELD AND DETENTION PERIOD

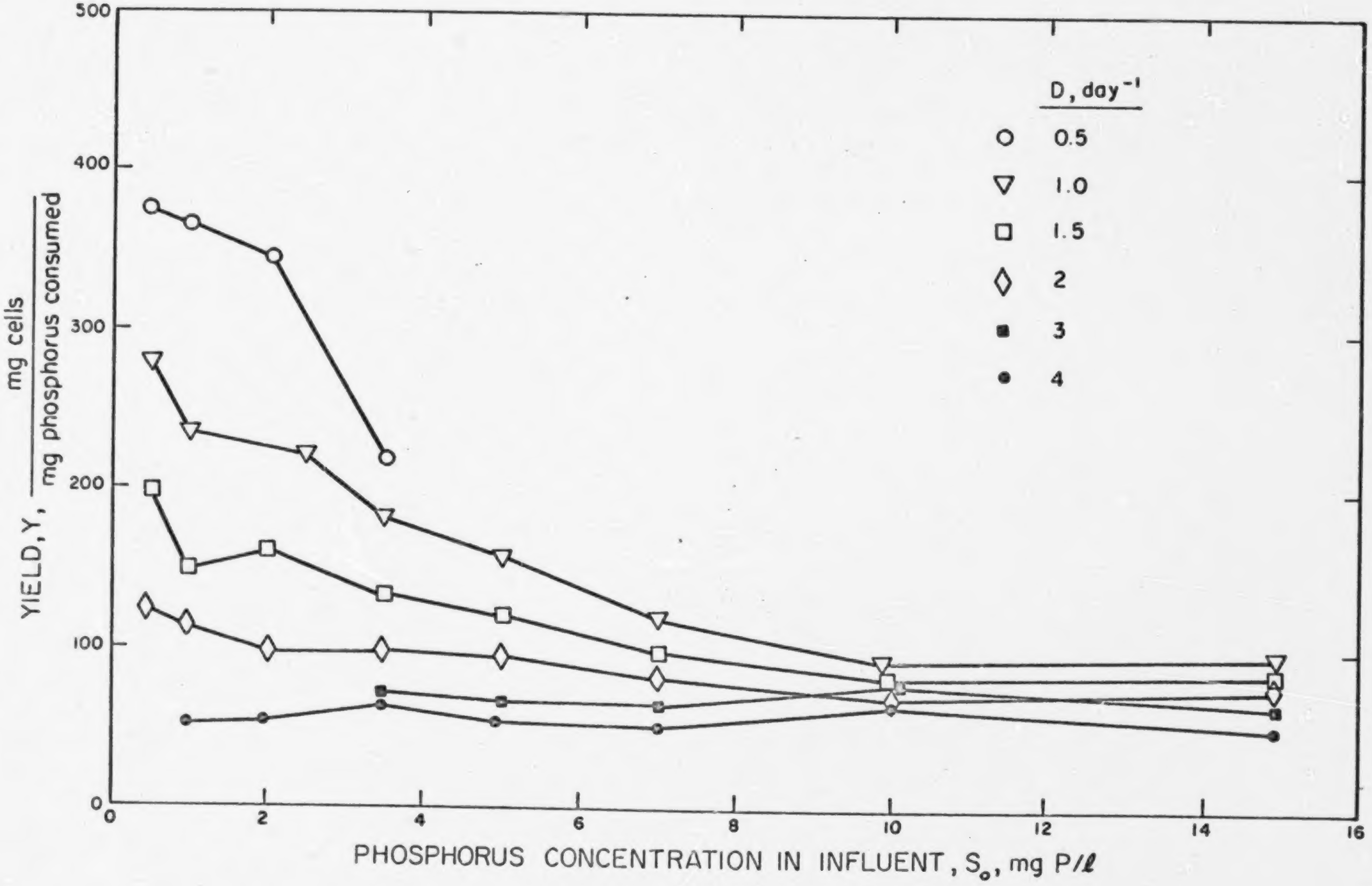

FIGURE 34. RELATIONSHIP BETWEEN YIELD AND PHOSPHORUS CONCENTRATION IN INFLUENT

per unit volume of the reactor is

$$P = \frac{Fx_1}{V} \tag{49}$$

or

$$P = Dx_1 = \frac{x_1}{\theta} \tag{50}$$

The cell production rates observed in the study are listed in column 11 of Table VIII and plots of the production rate as a function of dilution rate are given in Figures 35 and 36. With small phosphorus levels in the feed (up to 5 mg P/1), there was little variation in the production rate with the dilution rate. This is also indicated in Figures 37 and 38, which are plots of algal production against detention time.

With higher feed phosphorus, algal production increased with the dilution rate up to a dilution rate of 3 per day. Beyond this value, the change in the rate of production decreased. These behaviors can be explained by the variation of the algal concentration with the dilution rate and suggest that there is an optimum dilution rate for maximum cell production.

The relationship and between the phosphorus concentration in the feed and the rate of algal production is shown in Figures 39 and 40. Depending on the dilution rate, the algal production is proportional to the feed phosphorus up to a concentration of 5 to 7 mg P/1 and then levels off with further increase in the feed phosphorus.

It has been shown (207) that maximum algal production is associated with an optimum algal concentration. Plots of the algal production against algal concentration are shown in Figures 41-43.

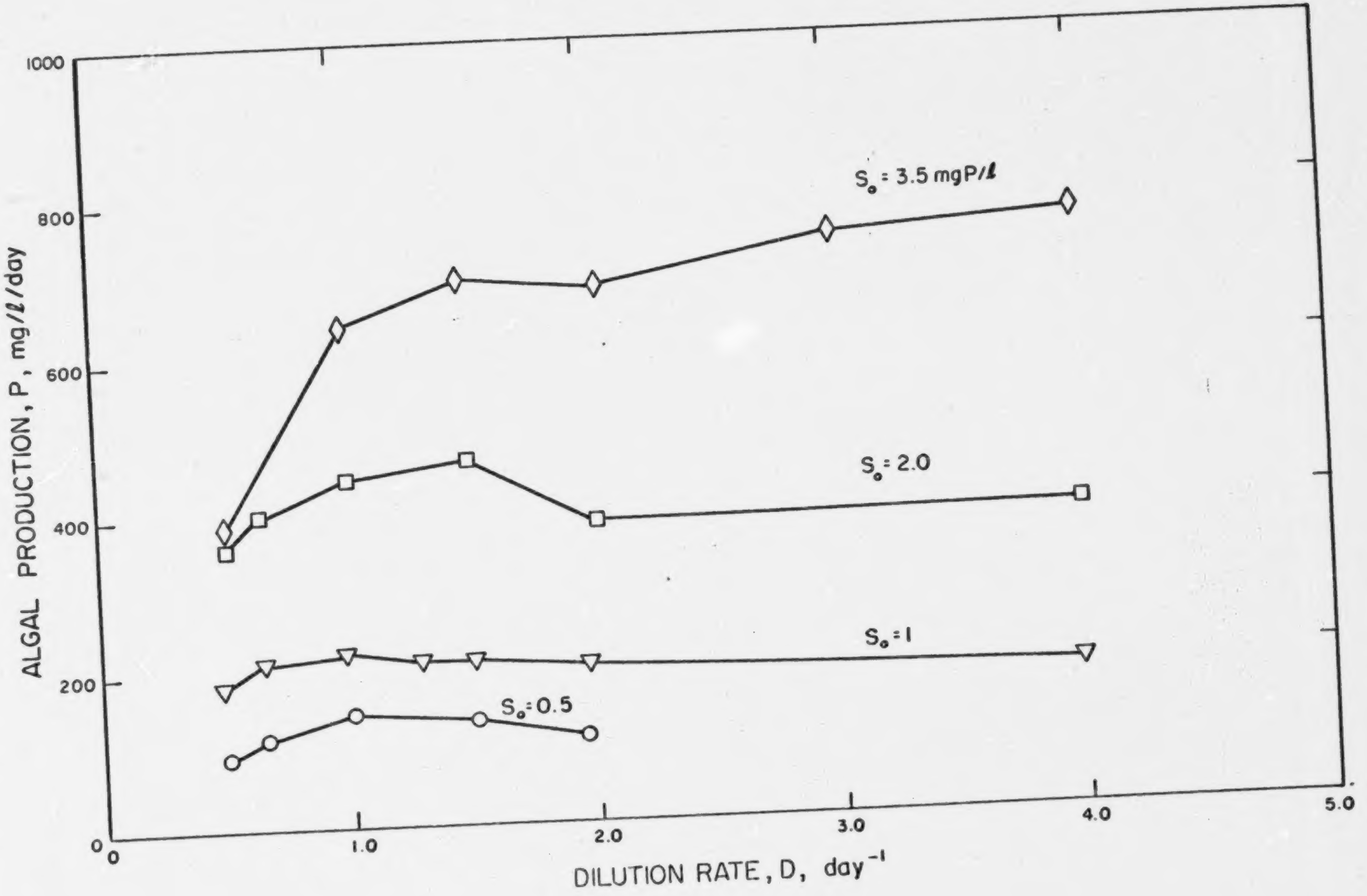

FIGURE 35. RELATIONSHIP BETWEEN ALGAL PRODUCTION AND DILUTION RATE

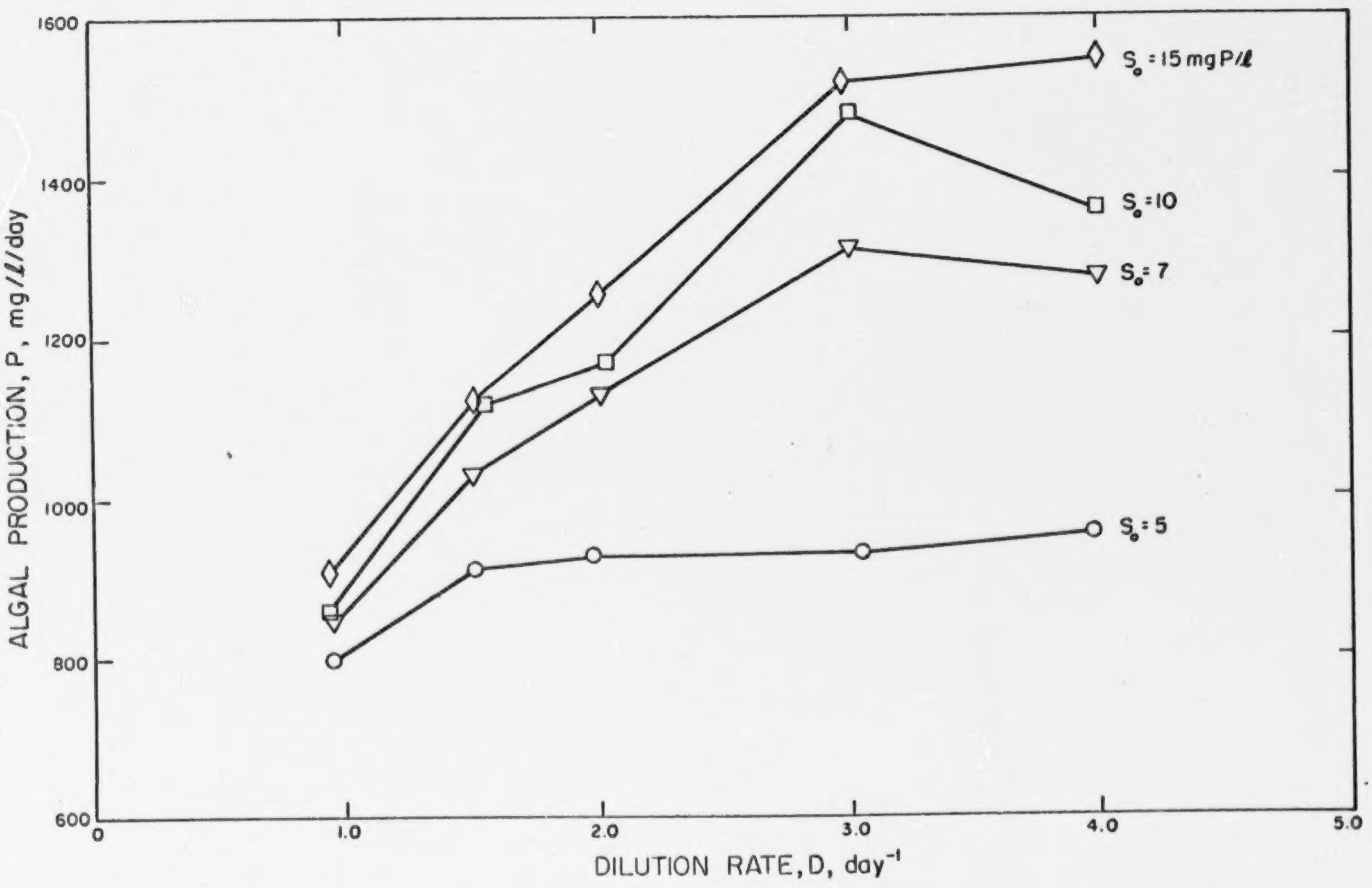

FIGURE 36. RELATIONSHIP BETWEEN ALGAL PRODUCTION AND DILUTION RATE

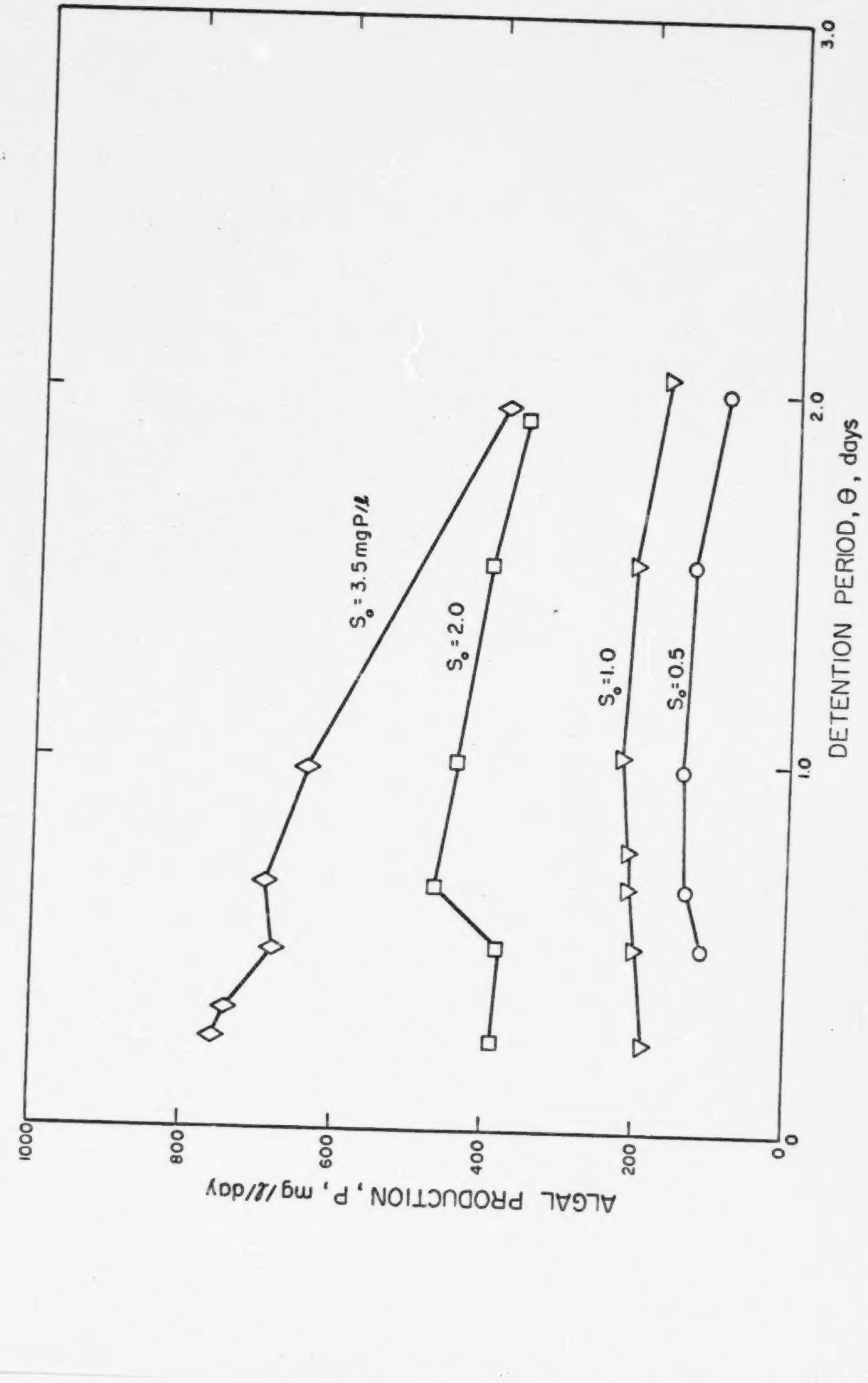

FIGURE 37. RELATIONSHIP BETWEEN ALGAL PRODUCTION AND DETENTION PERIOD

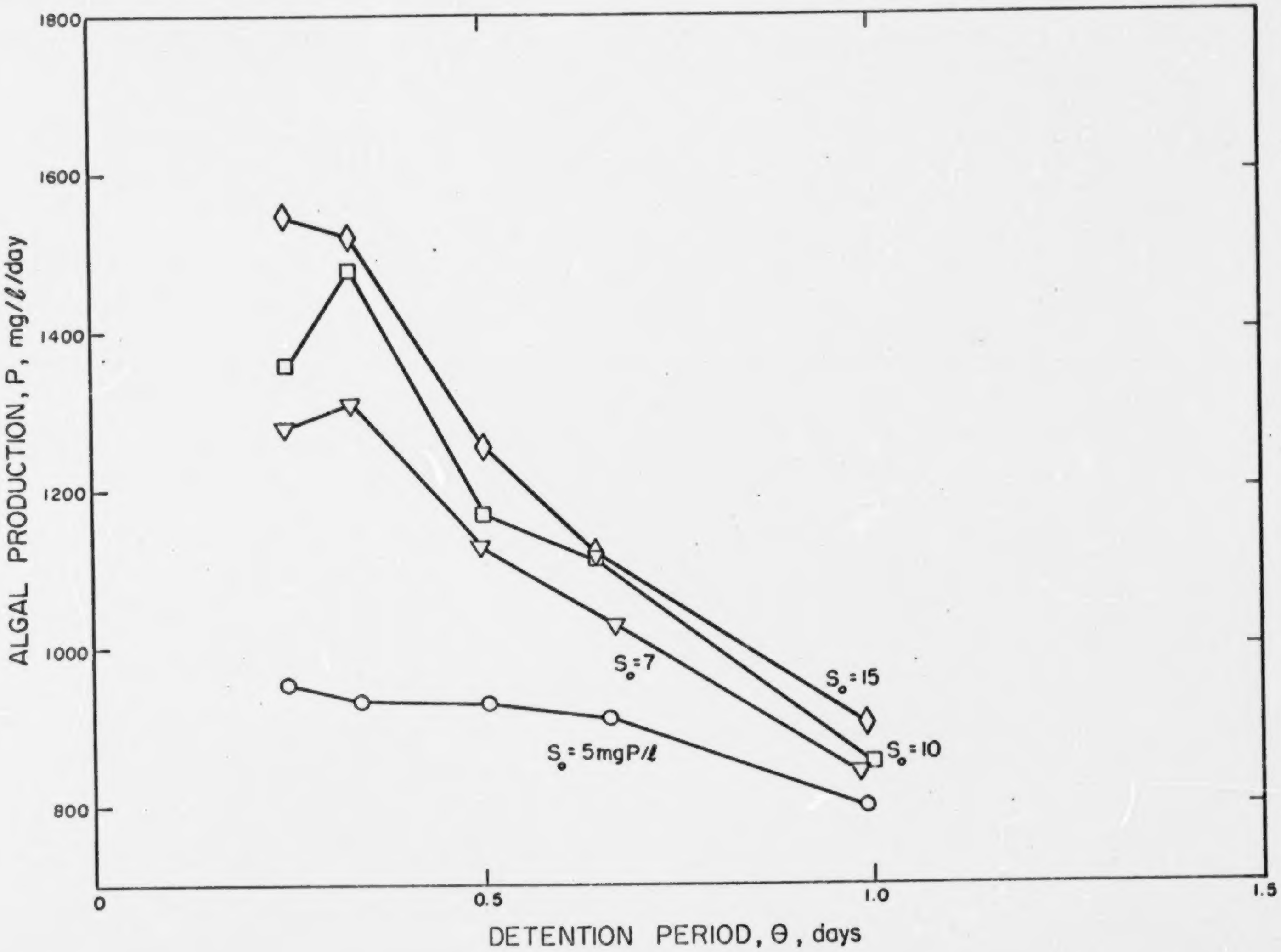

FIGURE 38. RELATIONSHIP BETWEEN ALGAL PRODUCTION AND DETENTION PERIOD

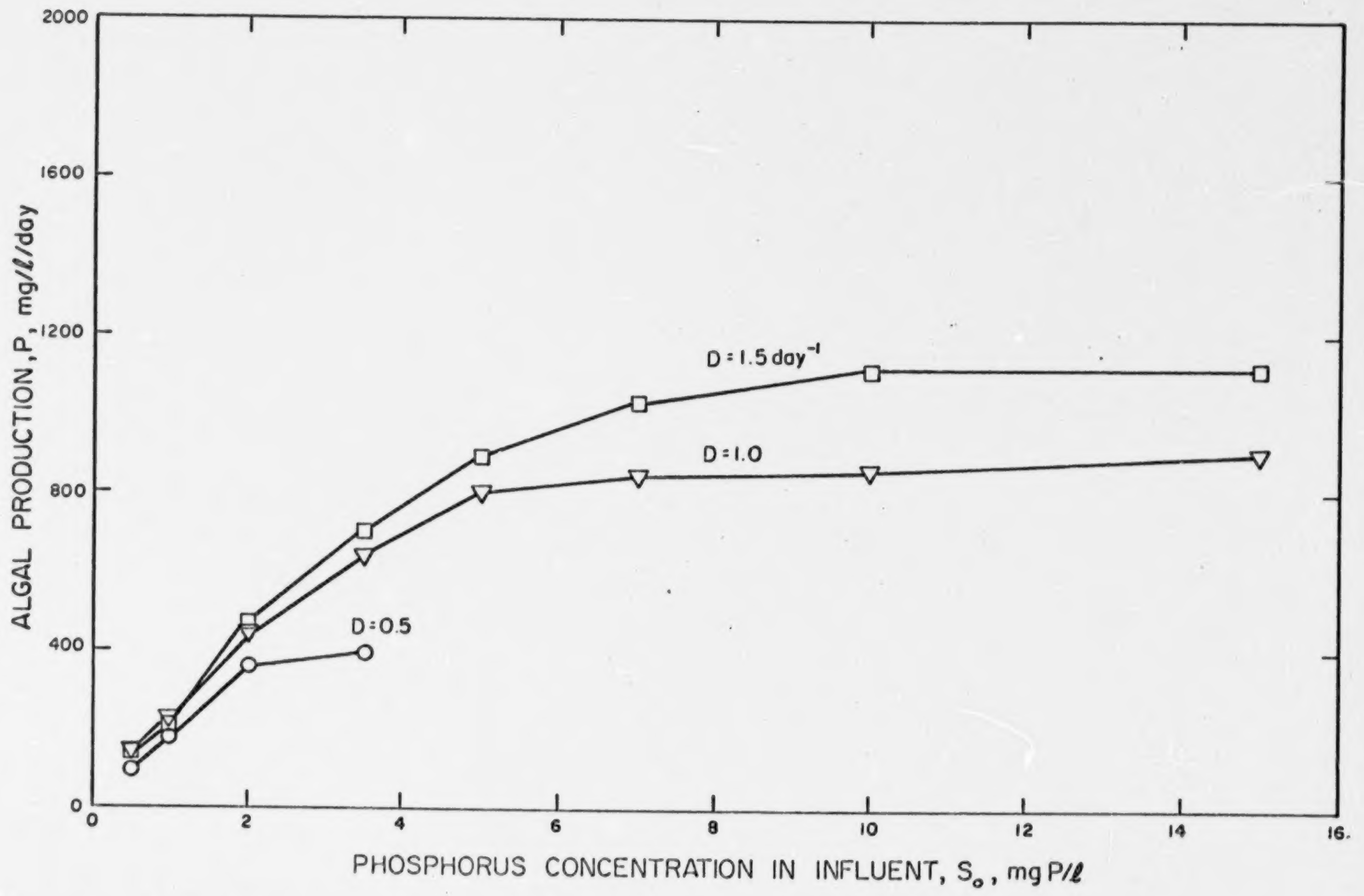

FIGURE 39. RELATIONSHIP BETWEEN ALGAL PRODUCTION AND PHOSPHORUS CONCENTRATION IN THE INFLUENT

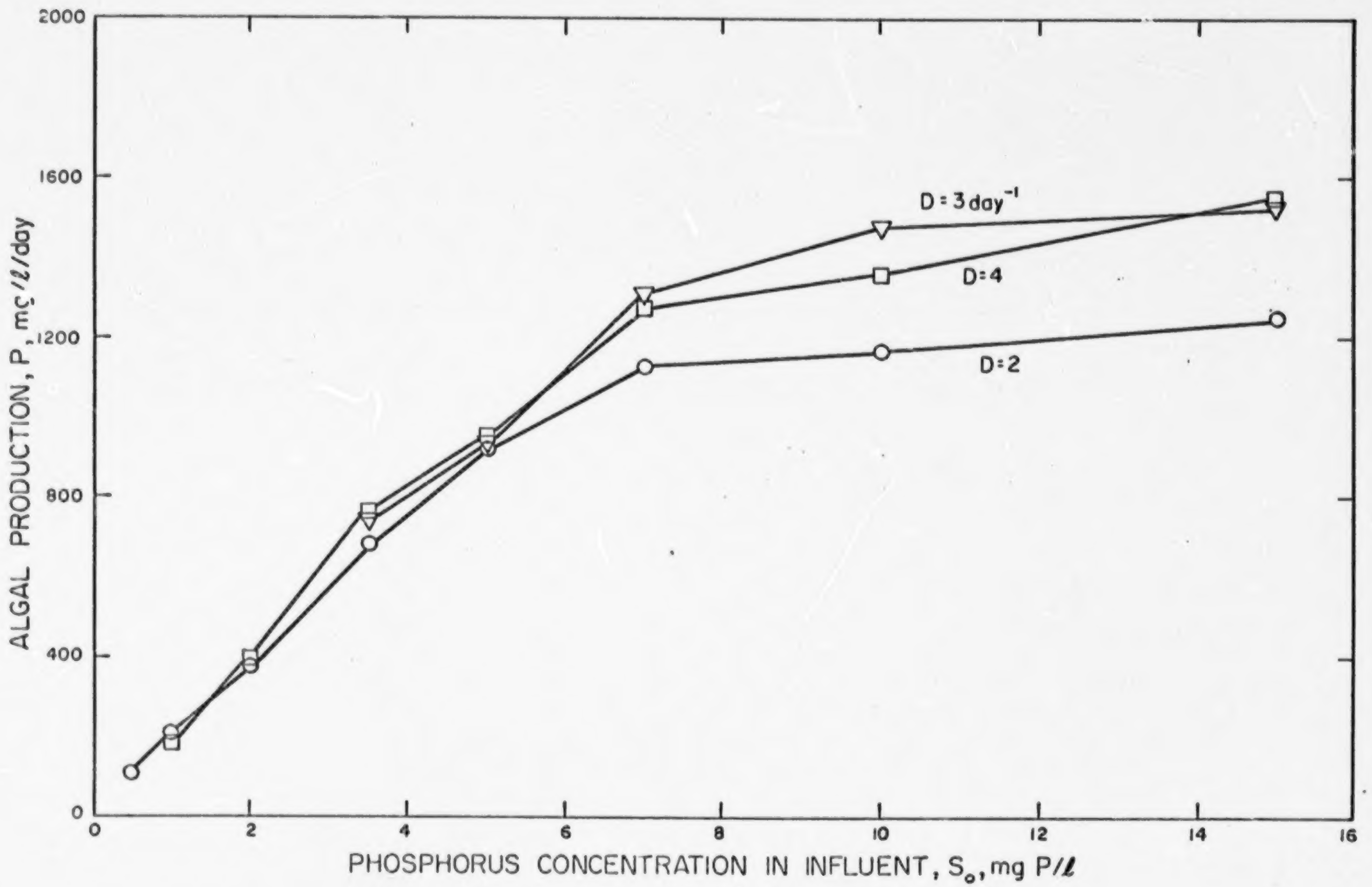

FIGURE 40. RELATIONSHIP BETWEEN ALGAL PRODUCTION AND PHOSPHORUS CONCENTRATION IN THE INFLUENT

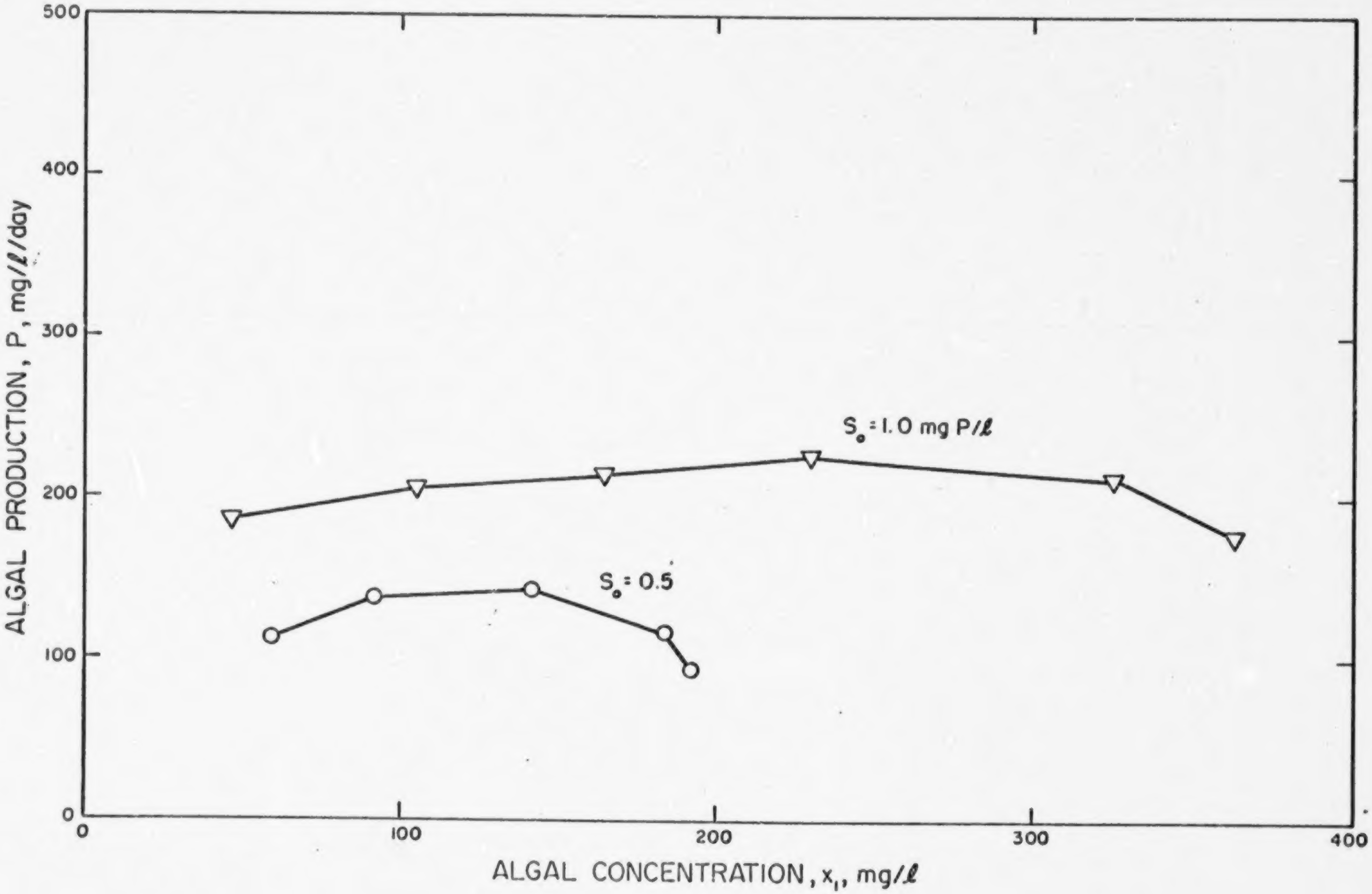

FIGURE 41. ALGAL PRODUCTION AS RELATED TO ALGAL CONCENTRATION

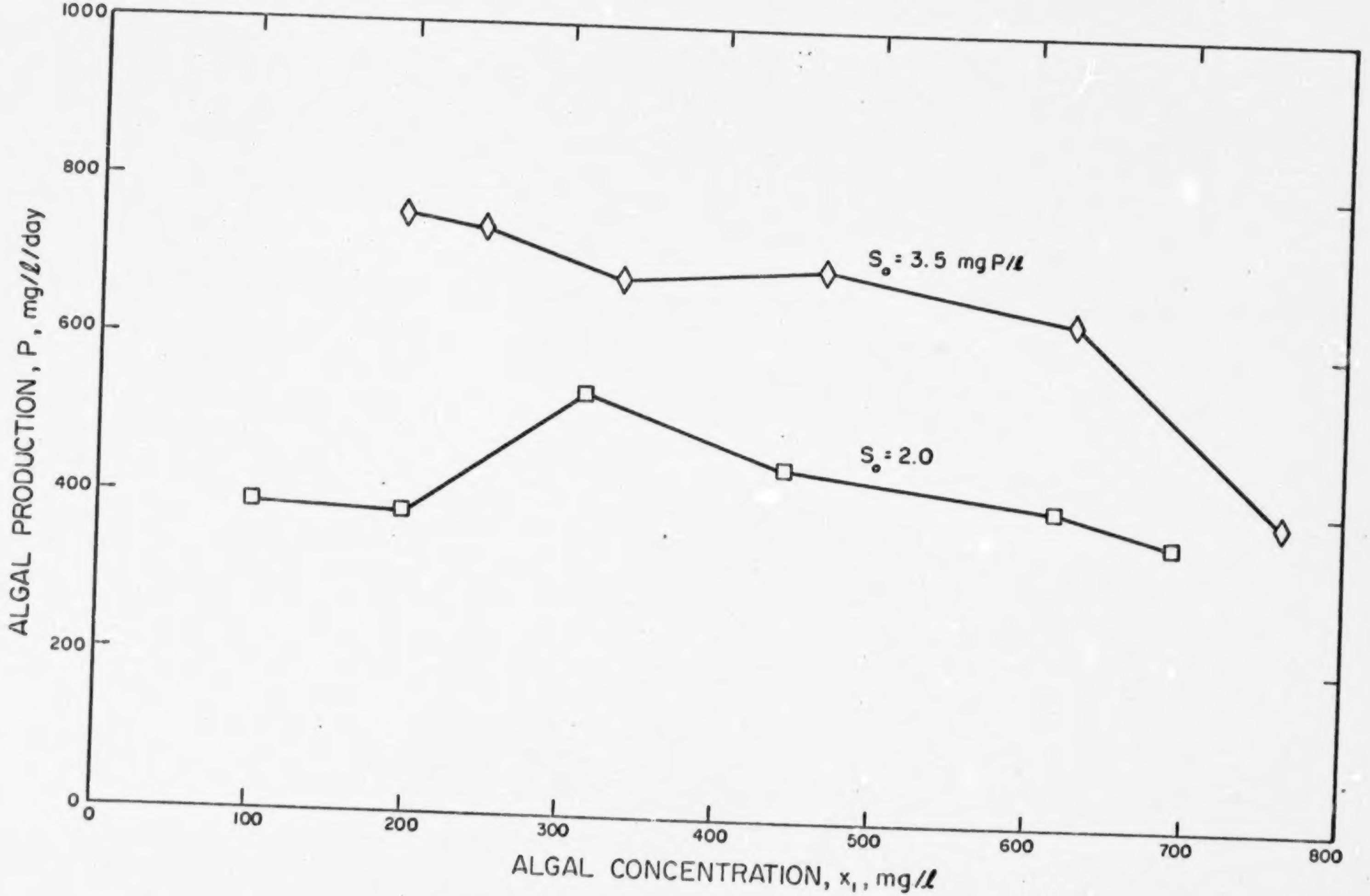

FIGURE 42. ALGAL PRODUCTION AS RELATED TO ALGAL CONCENTRATION

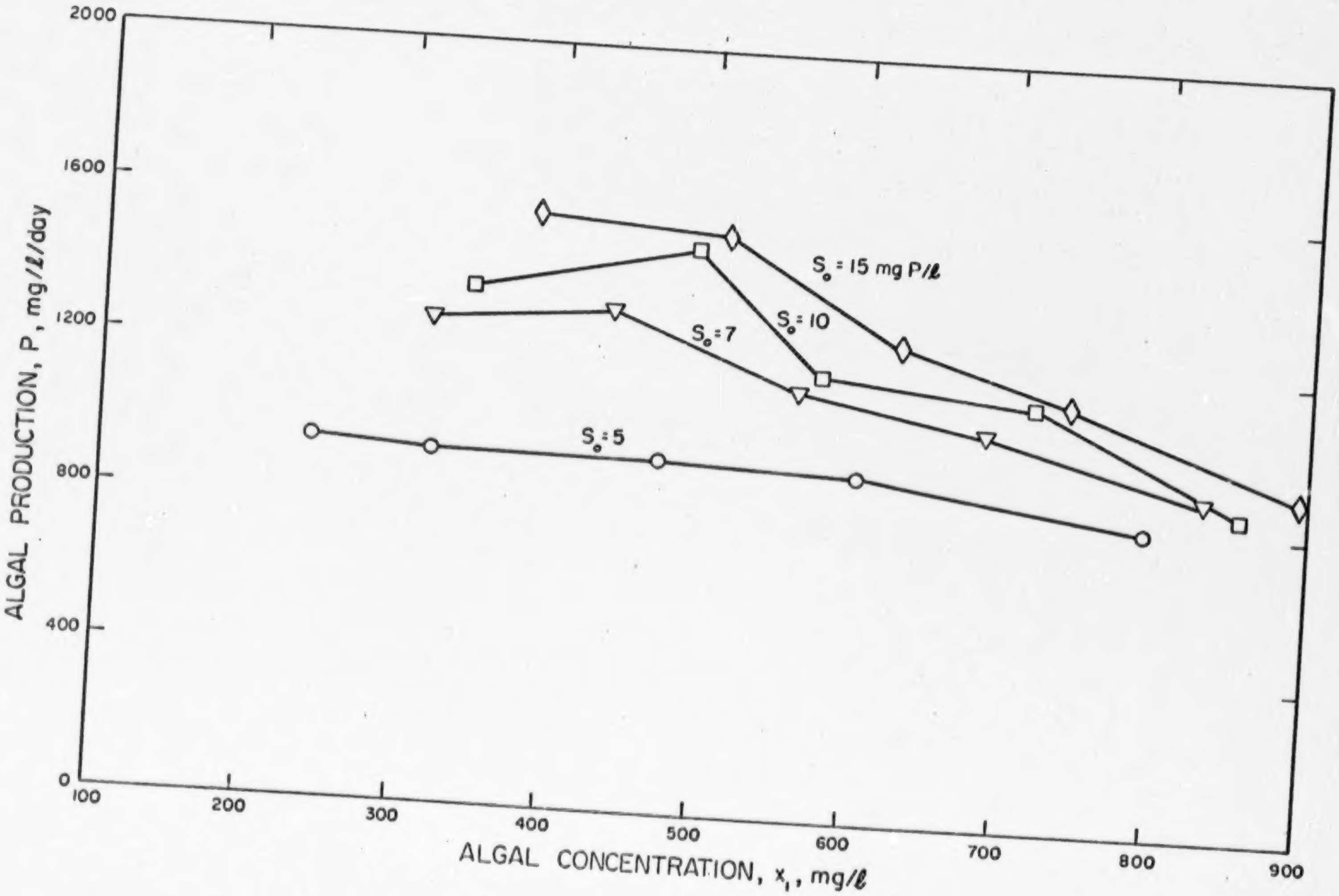

FIGURE 43. ALGAL PRODUCTION AS RELATED TO ALGAL CONCENTRATION

For the low feed phosphorus levels, the curves are similar in shape to those of algal production as a function of dilution rate, with only little variation in the production. The existence of an optimum concentration appears to be more distinct with the higher phosphorus concentrations in the feed.

EXPERIMENTS WITH CHLORELLA PYRENOIDOSA (EMERSON STRAIN)

Table X and Figures 44 to 51 show the results obtained from experiments using the Emerson strain of *Chlorella pyrenoidosa*. Only two sets of experiments, with phosphorus levels in the medium of 0.5 and 1.0 mg P/1, respectively, were carried out with this organism.

From Figures 44-47, it can be seen that the data were also well described by the Monod growth rate equation. The values of the kinetic parameters $\hat{\mu}$ and K_s determined from the experiments are given in Table XI. The maximum specific growth rates were about one-half and the saturation constant one-third to one-half of the corresponding values for the high-temperature strain.

In Figures 48 and 49 are plotted the algal concentration and the yield, respectively, as functions of the dilution rate. These characteristics varied in much the same manner with the dilution rate as those of the 71105 strain, but the values at a particular dilution rate were lower than the corresponding values for the 71105 strain.

Figure 50 shows the relationship between the production of algae and the dilution rate and Figure 51 the relationship between production and algal concentration. The data indicate that, in these cases, higher dilution rate and low culture concentration were more favorable for algal production.

TABLE X

SUMMARY OF GROWTH AND KINETIC DATA - EXPERIMENTS WITH C. pyrenoidosa (Emerson strain)

Expt. No.	Dilution Rate D day^{-1}	Detention Period θ days	Phosphorus in Feed S_o mg P/1	Phosphorus in Culture S_1 mg P/1	Algal Concentration x_1 mg/1	$\frac{1}{D}\left(=\frac{1}{\mu}\right)$	$\frac{1}{S_1}$	Cell Yield Y $\frac{\text{mg cells}}{\text{mg P}}$	Phosphorus Content of Cell Mass % of Dry Weight	Algal Production P mg/1/day	Efficiency of Phosphorus Removal %
B-I-1	0.25	4.00	0.510	0.0032	178	4.00	312	351	0.285	45	99.5
B-I-2	0.31	3.23	0.510	0.0039	169	3.23	257	334	0.299	52	99.3
B-I-3	0.505	1.98	0.510	0.0085	155	1.98	118	308	0.324	78	98.4
B-I-4	1.02	0.98	0.495	0.018	96	0.98	55.5	202	0.497	98	96.5
B-I-5	1.50	0.667	0.495	0.054	73	0.667	18.5	166	0.604	110	89.3
B-II-1	0.24	4.16	1.010	0.0037	348	4.16	270	348	0.288	84	99.0
B-II-2	0.33	3.02	1.010	0.0052	343	3.02	192	342	0.292	113	99.0
B-II-3	0.49	2.04	0.980	0.0078	324	2.04	128	334	0.300	159	99.2
B-II-4	0.65	1.54	0.980	0.014	269	1.54	71.5	278	0.359	181	98.6
B-II-5	1.01	0.99	1.015	0.021	176	0.99	47.5	177	0.564	178	98.0
B-II-6	1.52	0.656	1.015	0.047	137	0.656	21.3	141	0.707	208	95.3

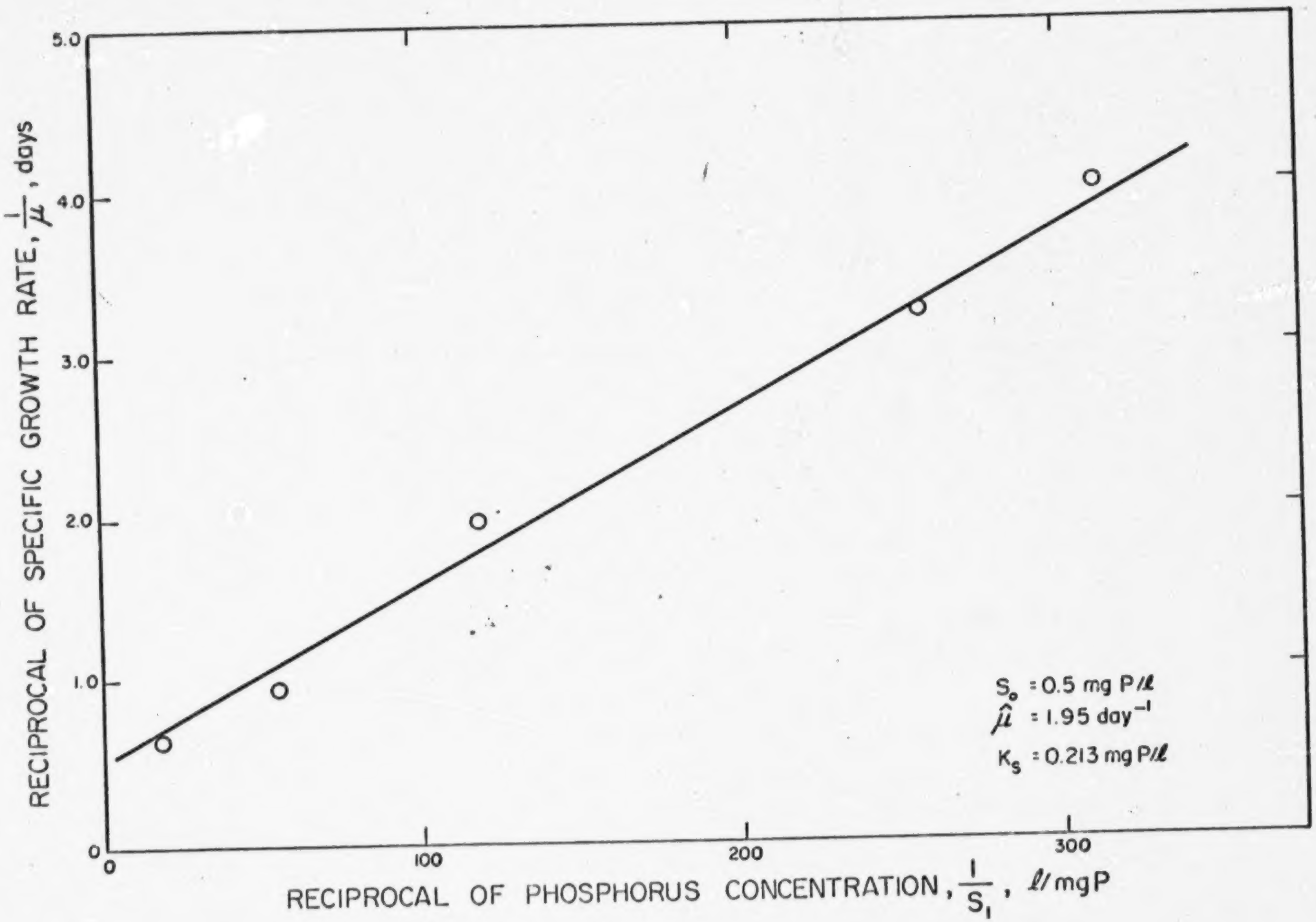

FIGURE 44. DETERMINATION OF THE MAXIMUM SPECIFIC GROWTH RATE, $\hat{\mu}$, AND SATURATION CONSTANT, K_s

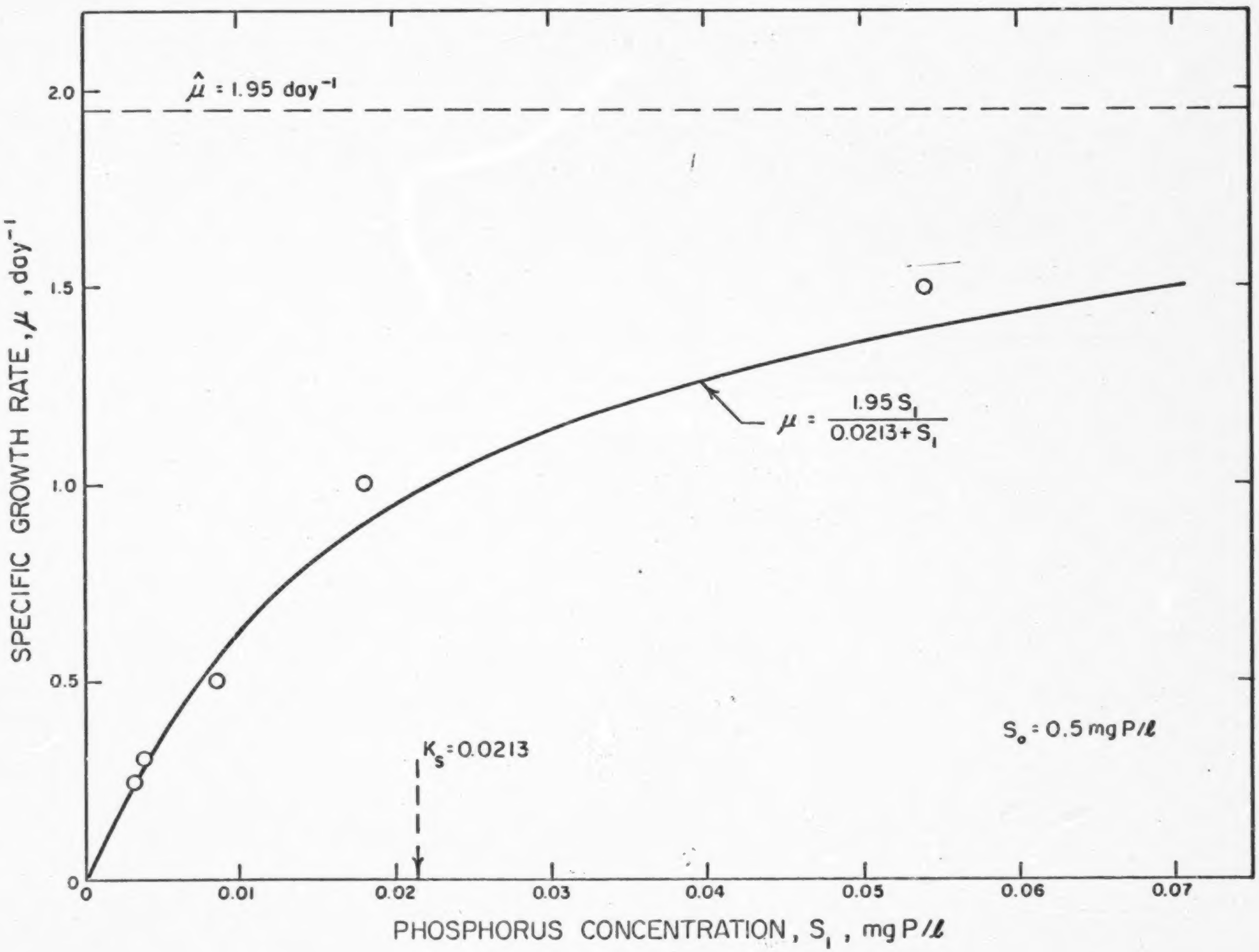

FIGURE 45. GROWTH RATE AS A FUNCTION OF PHOSPHORUS CONCENTRATION (C. Pyrenoidosa (Emerson str.); T = 25 ± 0.5 °C; pH = 7.5 ± 0.1)

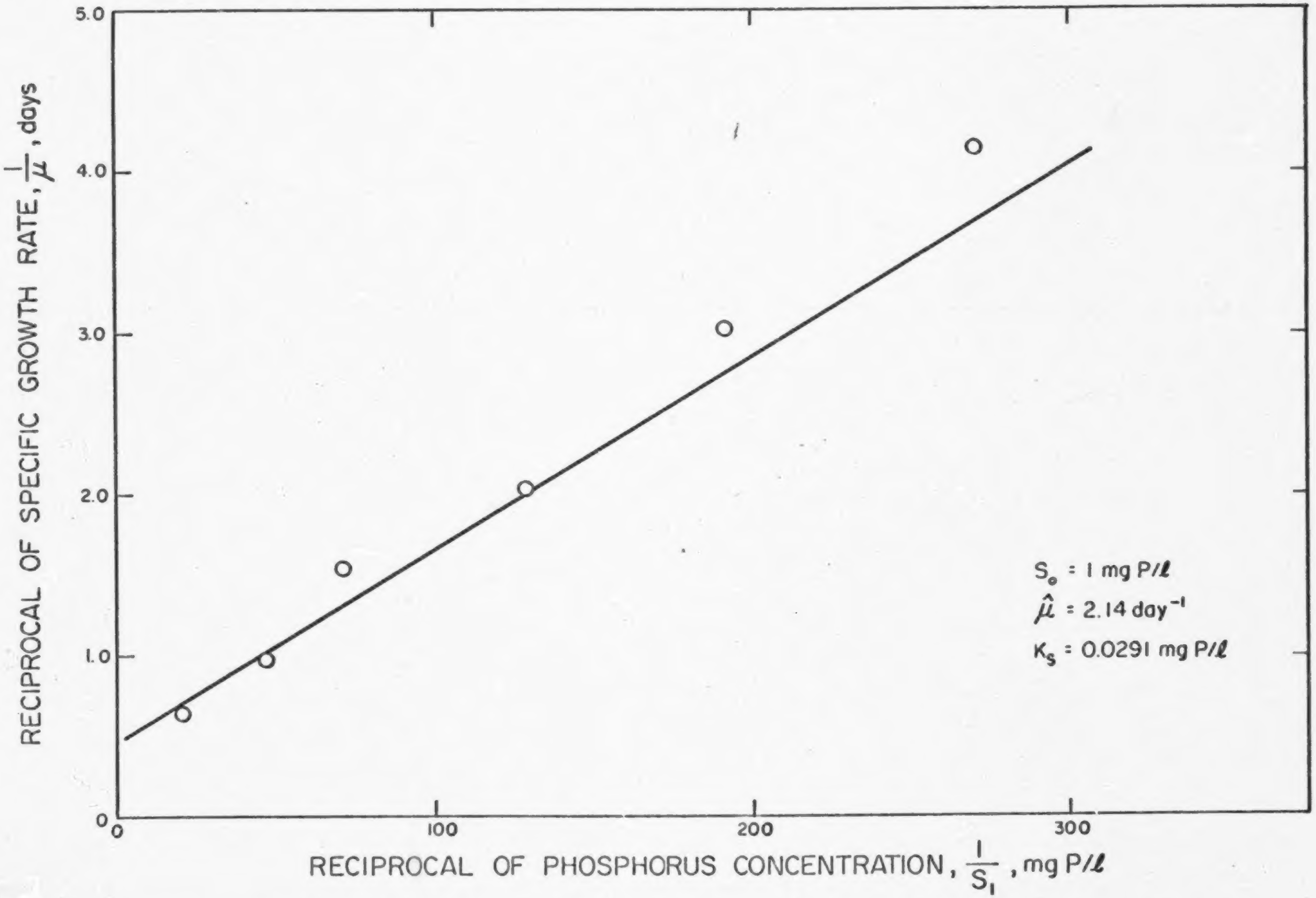

FIGURE 46. DETERMINATION OF THE MAXIMUM SPECIFIC GROWTH RATE, $\hat{\mu}$, AND SATURATION CONSTANT, K_s

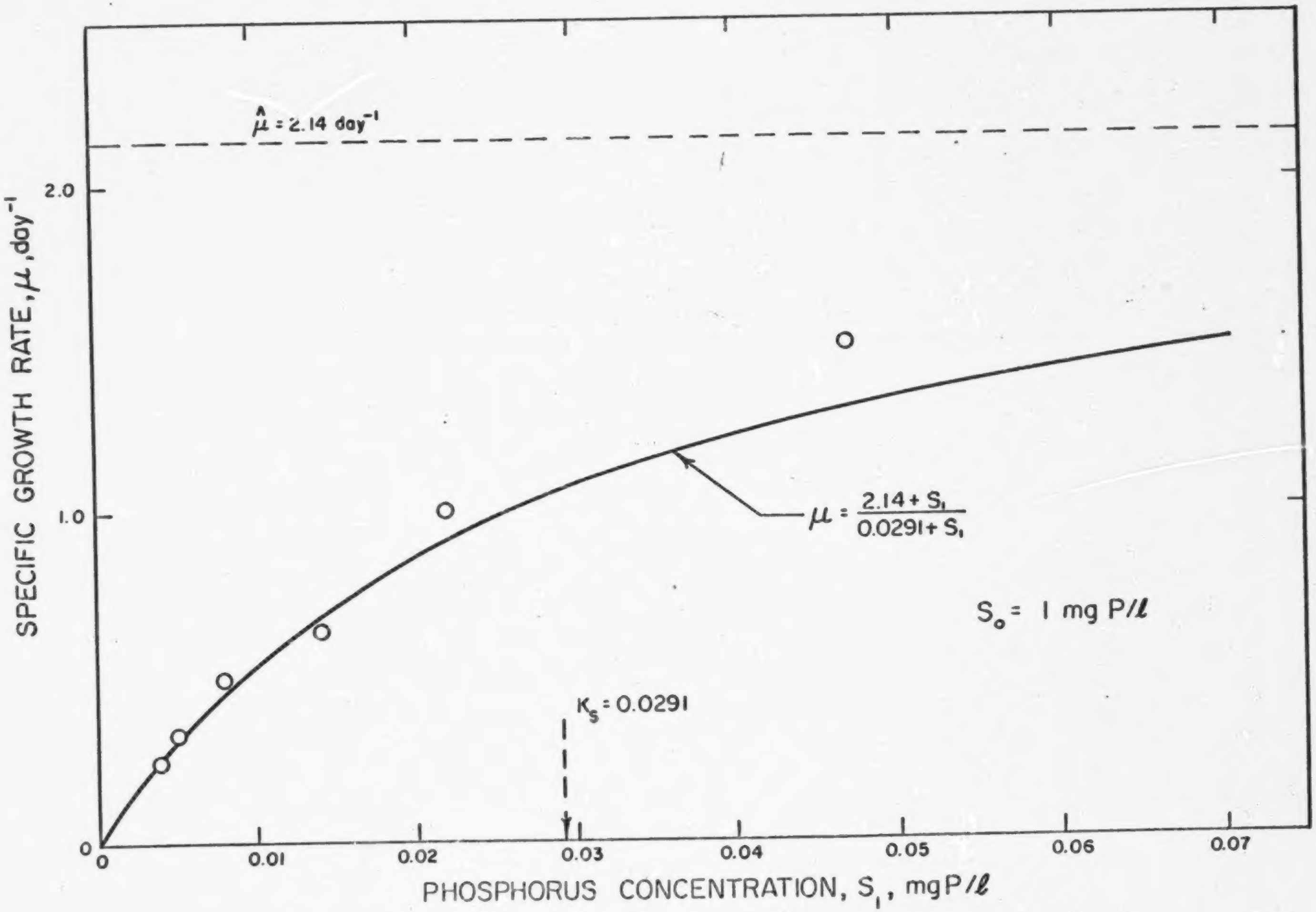

FIGURE 47. GROWTH RATE AS A FUNCTION OF PHOSPHORUS CONCENTRATION (*C. Pyrenoidosa* (Emerson str.); T = 25° ± 0.5 °C ; pH = 7.5 ± 0.1)

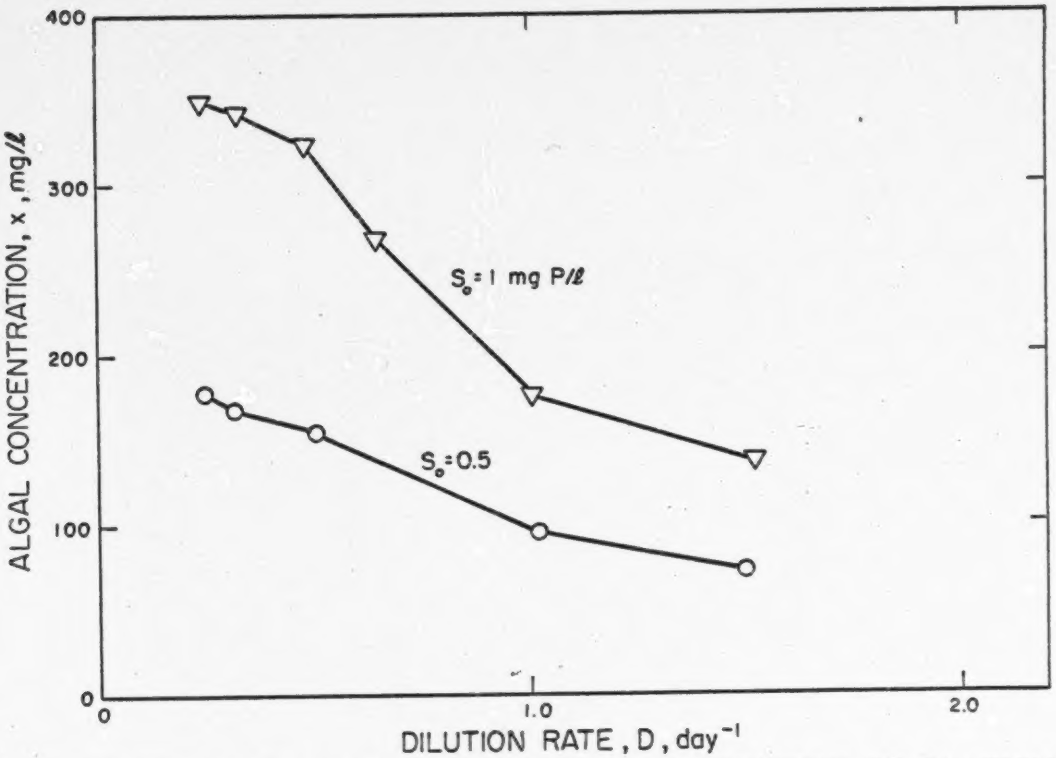

FIGURE 48. ALGAL CONCENTRATION AS A FUNCTION OF DILUTION RATE

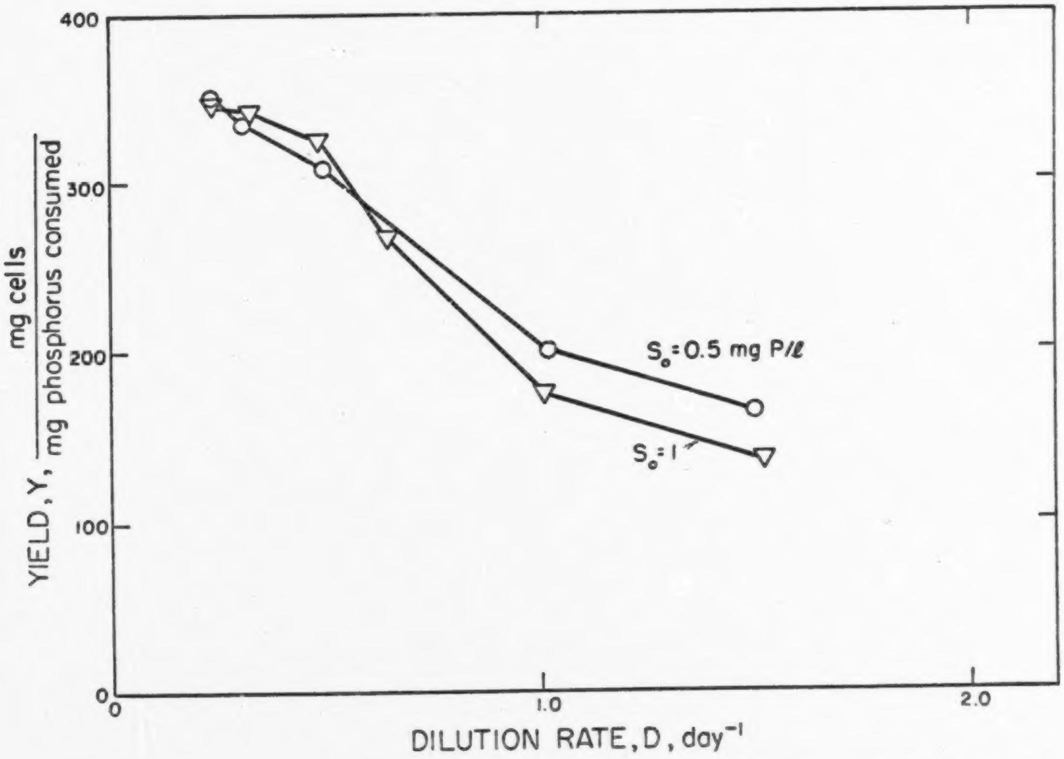

FIGURE 49. YIELD AS A FUNCTION OF DILUTION RATE

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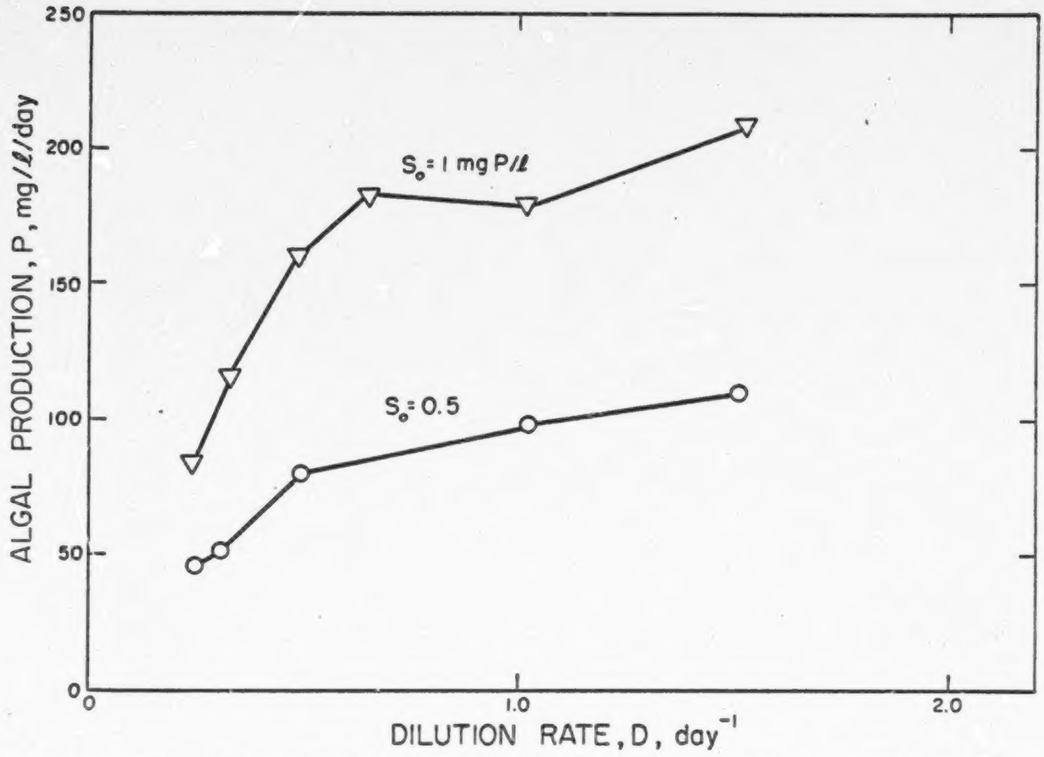

FIGURE 50. RELATIONSHIP BETWEEN ALGAL PRODUCTION AND DILUTION RATE

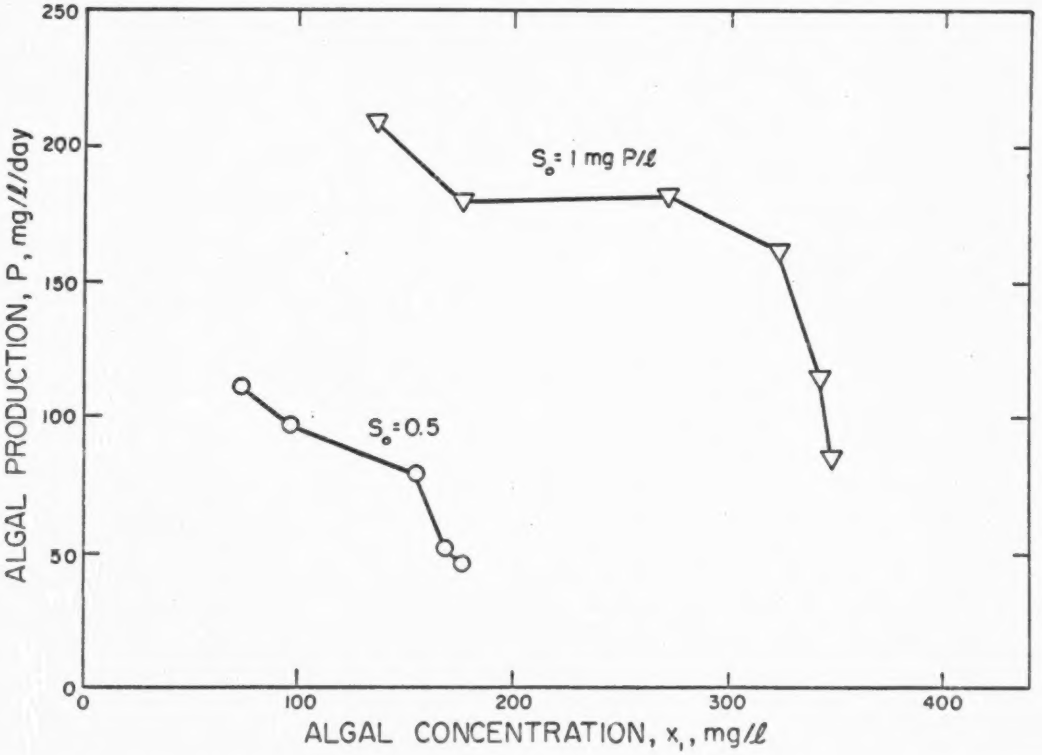

FIGURE 51. ALGAL PRODUCTION AS RELATED TO ALGAL CONCENTRATION

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TABLE XI

VALUES OF $\hat{\mu}$ AND K_s - EXPERIMENTS WITH C. Pyrenoidosa (Emerson str.)

Expt. No.	Phosphorus in Feed S_o mg P/l	Max. Specific Growth Rate $\hat{u}$ day^{-1}	Saturation Constant K_s mg P/l
B-I	0.5	1.95	0.0213
B-II	1.0	2.14	0.0291

EFFECT OF pH ON ALGAL GROWTH CHARACTERISTICS

The pH value is recognized as one of the most important factors affecting the growth and metabolism of microorganisms. To obtain an indication of the effect of this factor on algal growth and phosphorus absorption, an experiment consisting of runs at pH values of 7.0, 7.5, 8.0, and 8.25 was carried out. A dilution rate of 1 day^{-1} and a feed phosphorus concentration of 1 mg P/l were used. The strain 71105 of C. pyrenoidosa was employed as the culture organism and a temperature of 39 ± 0.5°C was used. The results are given in Table XII and plots of the equilibrium cell concentration, cell yield, and phosphorus content of the cell mass are shown in Figure 52.

From the data, it can be seen that the lower pH provided a more favorable environment than higher pH values for the culture organism used. At a pH value of 7.0, the equilibrium cell concentration was 11 per cent greater than the value at pH 7.5, twice as much as that at pH 8.0, and more than 3 times the concentration attained at pH 8.25. The phosphorus in the feed was almost completely removed in all the runs but the residual medium phosphorus concentrations were greater at the higher pH values than at the lower ones. Since the cell yield is proportional to the cell concentration, the yield also declined as the pH was raised. The phosphorus content of the cell mass increased as the pH rose indicating that the algae were probably less efficient in the utilization of phosphorus for cell conversion at the higher pH values.

EFFECT OF TEMPERATURE ON ALGAL GROWTH CHARACTERISTICS

An experiment was also performed to observe the influence of temperature on the culture characteristics of the 71105 strain of

TABLE XII

EFFECT OF pH ON ALGAL GROWTH CHARACTERISTICS*

pH Value	Dilution Rate D day^{-1}	Phosphorus in Feed S_o mg P/1	Phosphorus in Culture S_1 mg P/1	Algal Concentration x_1 mg/1	Cell Yield Y $\frac{mg\ cells}{mg\ P}$	Phosphorus Content of Cell Mass % of Dry Weight
7.0	1.0	1.0	0.017	258	262	0.38
7.5	0.98	1.0	0.020	230	235	0.43
8.0	1.0	1.0	0.029	172	177	0.57
8.25	1.0	1.01	0.045	81	84	1.19

*C. pyrenoidosa, strain 71105; Temp. = 39 ± 0.5 °C

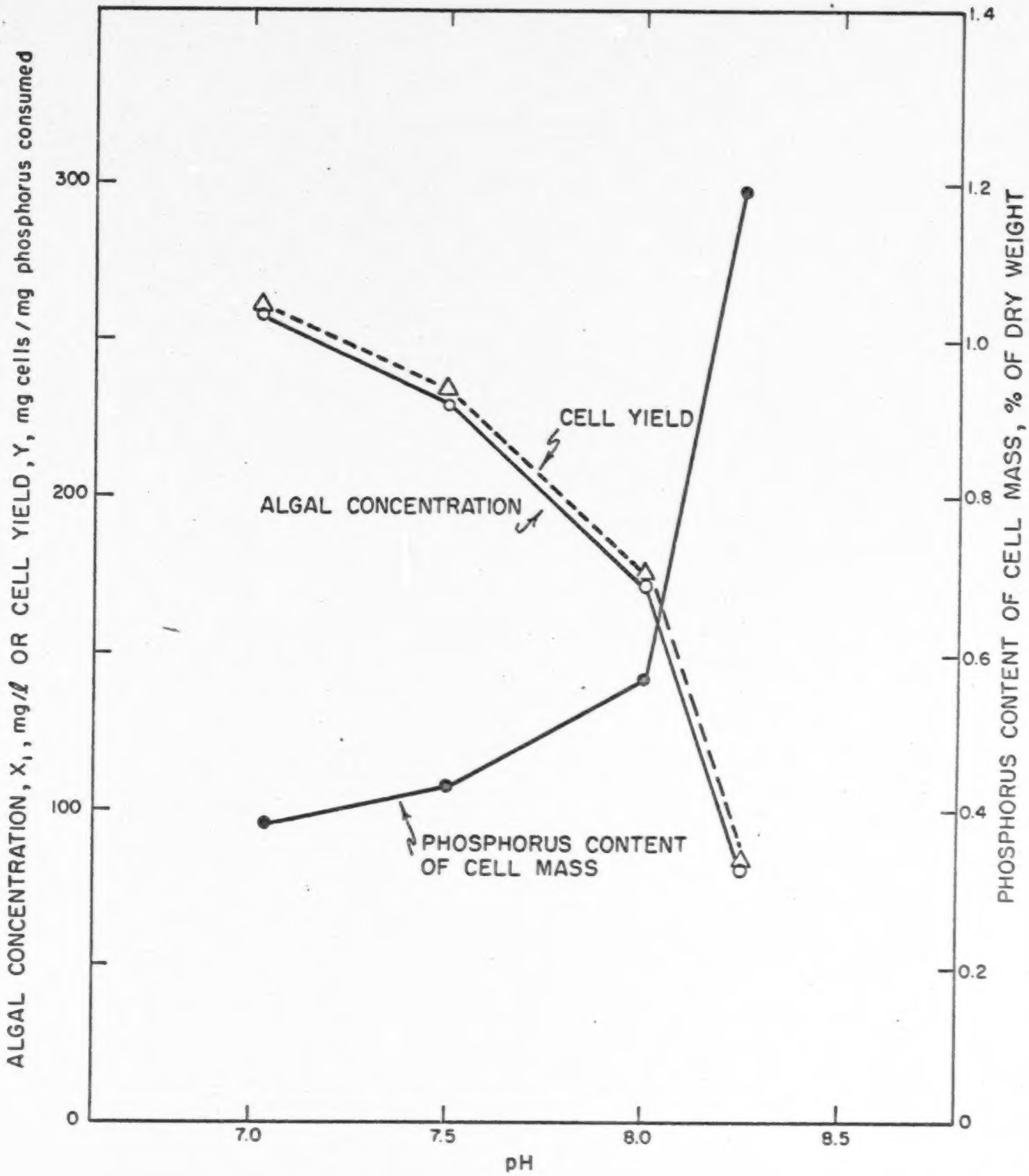

FIGURE 52. EFFECT OF pH ON ALGAL GROWTH CHARACTERISTICS (*C. Pyrenoidosa* 71105, T = 39 ± 0.5 °C)

C. pyrenoidosa by running steady-state continuous cultures at 30, 35, 39, and 41°C. The experiment employed also a dilution rate of 1 day^{-1} and a phosphorus concentration in the feed of 1 mg P/l. The pH was 7.5.

The data given in Table XIII and Figure 53 clearly show that temperature exerts a significant effect on the characteristics of the culture. At 41°C., the culture was washed out indicating that this temperature was intolerable for the organism. From the data obtained, the most favorable temperature was 39°C. and the farther the culture temperature was from this value the lower were the equilibrium cell concentration and cell yield. Phosphorus removal was also high in all the runs, except in the run at 41°C., but was less at the lower than at the higher temperatures. The phosphorus content of the cell mass was again higher at the less favorable conditions.

EFFICIENCY OF PHOSPHORUS REMOVAL

The ability or capacity of any technique to remove an undesirable component of waste water is conventionally expressed in terms of efficiency of removal. Phosphorus removal efficiencies calculated from the results of the study are listed in column 12 of Tables VIII and X. As can be noted, for the 71105 strain, the removal efficiencies were high in all the runs with up 7 mg P/l phosphorus concentration in the feed. The removal efficiencies ranged from almost complete removal at a dilution rate of 0.5 day^{-1} to 87 per cent at a dilution rate of 4 day^{-1}. For the Emerson strain, the removal efficiencies ranged from almost complete removal at a dilution rate of 0.5 day^{-1} to 89 per cent at a dilution rate of 1.5 day^{-1}.

TABLE XIII

EFFECT OF TEMPERATURE ON ALGAL GROWTH CHARACTERISTICS*

Temperature $^\circ C$	Dilution Rate D day^{-1}	Phosphorus in Feed S_o mg P/1	Phosphorus in Culture S_1 mg P/1	Algal Concentration x_1 mg/1	Cell Yield Y $\frac{\text{mg cells}}{\text{mg P}}$	Phosphorus Content of Cell Mass % of Dry Weight
30	1.02	1.02	0.09	98	105	0.95
35	1.02	1.01	0.042	127	131	0.76
39	0.98	1.0	0.020	230	235	0.43
41	1.01	1.04	1.04	0	0	

*C. pyrenoidosa, strain 71105; pH = 7.5

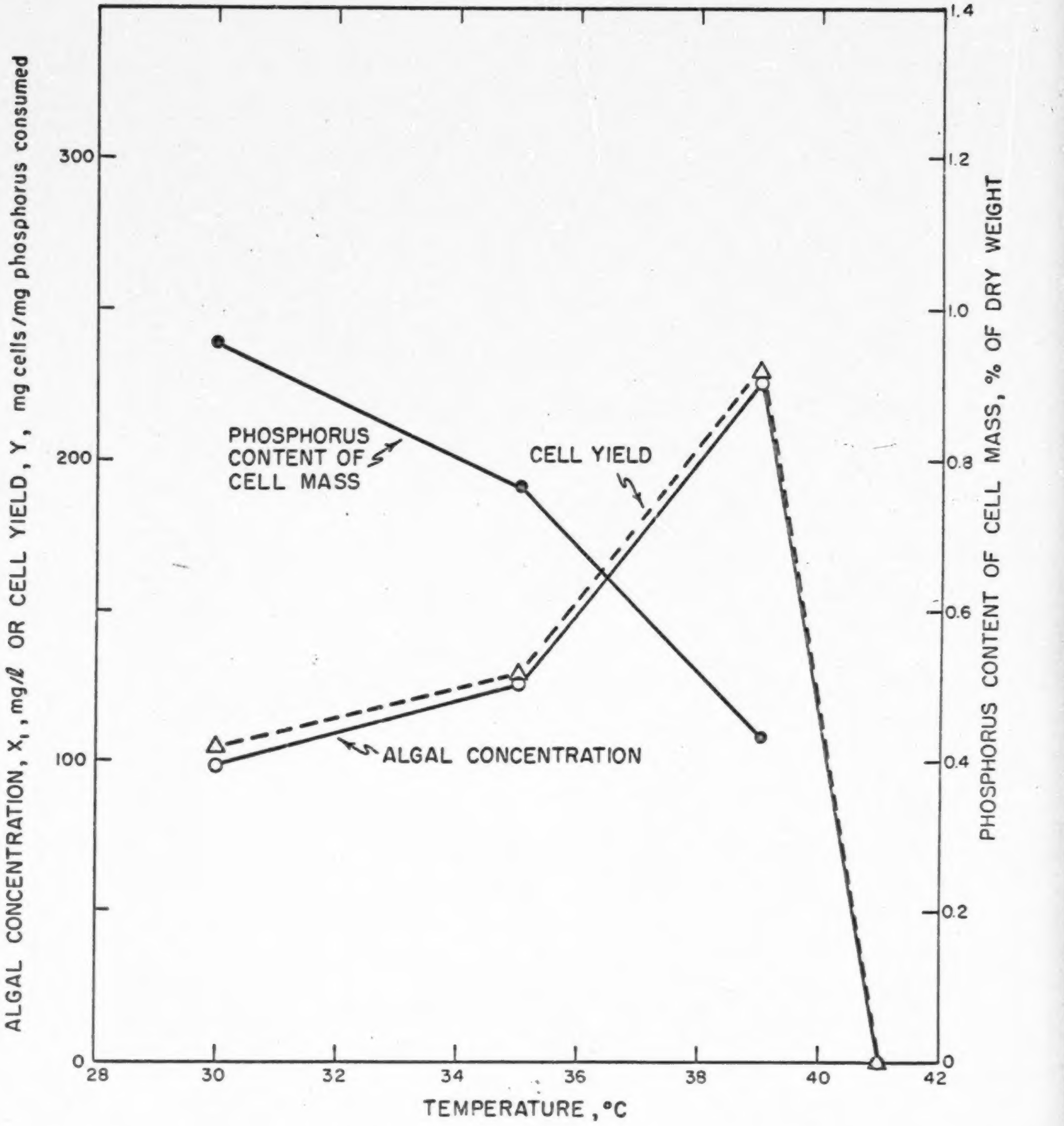

FIGURE 53. EFFECT OF TEMPERATURE ON ALGAL GROWTH CHARACTERISTICS (C. Pyrenoidosa 71105, pH = 7.5)

DISCUSSION

The purpose of this investigation was to study the kinetics and characteristics of phosphorus-limited algal cultures. A laboratory study was carried out with chemostatic continuous cultures using two strains of Chlorella pyrenoidosa, the 71105 and the Emerson strains. The results indicate that the growth rate when phosphorus is a limiting nutrient can be characterized by the Monod growth equation. With the culturing conditions used, an average value of 4.19 per day for the maximum specific growth rate, $\hat{\mu}$, was calculated for the strain 71105 of Chlorella pyrenoidosa. Values of the maximum specific growth rate obtained for the Emerson strain of the same organism were about one-half of those of the strain 71105. The magnitude of the saturation constant, K_s, for both strains was small, with an average value of 0.0582 mg P/l for the strain 71105 and about one-half to one-third of this value for the Emerson strain.

The values of the maximum specific growth rate determined in this study are lower than those in the literature. Sorokin (206, 209) has reported a maximum specific growth rate equivalent to 6.38 per day for the strain 71105 and 2.15 per day for the Emerson strain at light saturation and at the optimum temperatures for growth of the organisms. The differences between the values obtained in this study and those reported by Sorokin may probably be explained by the differences in the culturing conditions used and the techniques with which maximum specific growth rates were determined as well as the effects of other environmental factors.

The steady state culture concentration decreased with increase in the dilution rate. This behavior is similar to that observed by other workers for algae grown in chemostatic continuous cultures (191, 197, 207).

Cell yield is related to the consumption of substrate or nutrient and reflects the cellular content of the organisms for the nutrient. The cell yield or yield coefficient has been found to be fairly constant over a wide range of dilution rates under given culturing conditions for pure cultures of bacteria and other organisms and various single substrates or nutrients. A constant yield coefficient is commonly assumed in biological studies even for mixed cultures and complex substrates, although it is recognized that the yield coefficient could be variable. The results of this study showed the latter to be the case. The yield decreased with increase in the dilution rate and exhibited the same pattern as the cell concentration in relation to the dilution rate.

A varying yield factor indicates a variable phosphorus content of the cell mass. The finding of a changing cell yield in this study and thus, a changing phosphorus content of the algal cells, is not inconsistent with the reports in the literature. Many workers have observed a varying phosphorus consumption by algae. The phosphorus content per unit cell mass determined in this study ranged from 0.268 to 1.94 per cent of the dry weight. The values are within the range of values reported in the literature. The phosphorus content was larger at higher than at lower dilution rates indicating either or both a greater requirement for the nutrient and storage of phosphorus at the high dilution rates.

The consumption of phosphorus by algae in excess of 'normal' amounts needed for growth and metabolism has been noted by a number of investigators (17, 109, 110, 111). In some recent work (17, 210), the 'normal' phosphorus requirement and phosphorus content have been indicated to be in the neighborhood of 1 per cent of the dry weight for certain algae. Borchardt and Azad (17), in studies of phosphorus uptake by uni-algal and mixed cultures of Chlorella and Scenedesmus with a turbidostat type of continuous culture, concluded that the normal phosphorus requirement of the algae was 3 per cent (as PO_4^{-3}, approximately 1 per cent as P) of the dry weight and a phosphorus content above 3 per cent (as PO_4^{-3}) up to 9 per cent (as PO_4^{-3}) of the dry weight was luxury uptake. Moore and Fruh (210) studied surplus phosphorus uptake by axenic and non-axenic cultures of Chlorella pyrenoidosa (Emerson strain), Scenedesmus obliquus, and Anacystic nidulans under batch conditions in the laboratory and reported that the normal requirement for phosphorus was approximately 0.7 to 1.0 per cent of the cellular dry weight. There is a question, however, whether this value or range of values for the normal phosphorus requirement or phosphorus content is true for other organisms or strains or other culturing conditions. The normal or the range of normal phosphorus content could be different at a different temperature, pH, or other non-nutrient factor for the same organism or strain. For these reasons, it is difficult to state that luxury consumption of phosphorus has taken place in the runs where the phosphorus content of the cell mass was greater than 1 per cent in this study.

In studies with nitrogen as a limiting nutrient, Shelef et al. (207) noted that the yield of algae with respect to nitrogen was also not a constant but was strongly influenced by the nitrogen concentration in the culture. The yield-nitrogen concentration relationship exhibited a monotone-decreasing concave curve almost asymptotic to a certain value of the yield. The general shape of the curve resembled that of the growth rate in the sense that the yield approximated a constant value at saturation levels of the limiting nutrient.

The plots in Figures 54 and 56 for the 71105 and Emerson strains, respectively, show curves with similar shapes to that described above. The shape of the curves suggested a linear relationship between the yield and the reciprocal of the phosphorus concentration. Plots of the yield as a function of the reciprocal of the phosphorus concentration are shown in Figure 55 for the 71105 strain and in Figure 57 for the Emerson strain. The data points are quite scattered but they do indicate a trend of increasing yield with increasing $1/S_1$. In the absence of a more definitive relationship and for practical applications, the data can probably be assumed to be expressible by a straight line with which approximate values of the yield can be predicted. Thus, the yield can be estimated by the following equation:

$$Y = C_y \left(\frac{1}{S_1}\right) + Y_m \tag{51}$$

where

C_y = the slope of the line

Y_m = the y-intercept, corresponds to the minimum yield.

With the above relationship, the steady state cell concentration as well as the cell production rate can also be predicted. The equation for the cell concentration would be

$$x_1 = Y(S_o - S_1) = (S_o - S_1)\left(\frac{C_y}{S_1} + Y_m\right) \tag{52}$$

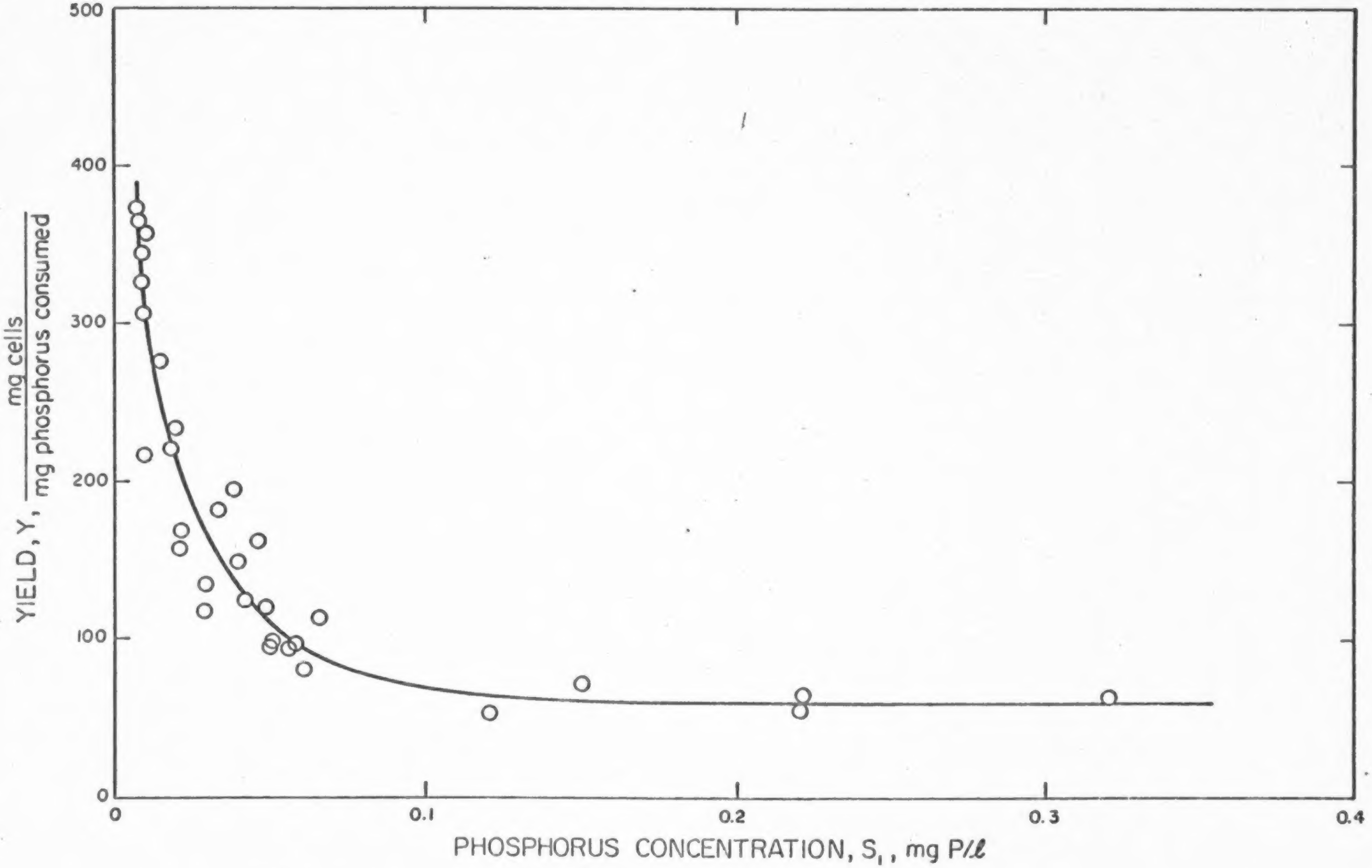

FIGURE 54. YIELD AS A FUNCTION OF PHOSPHORUS CONCENTRATION

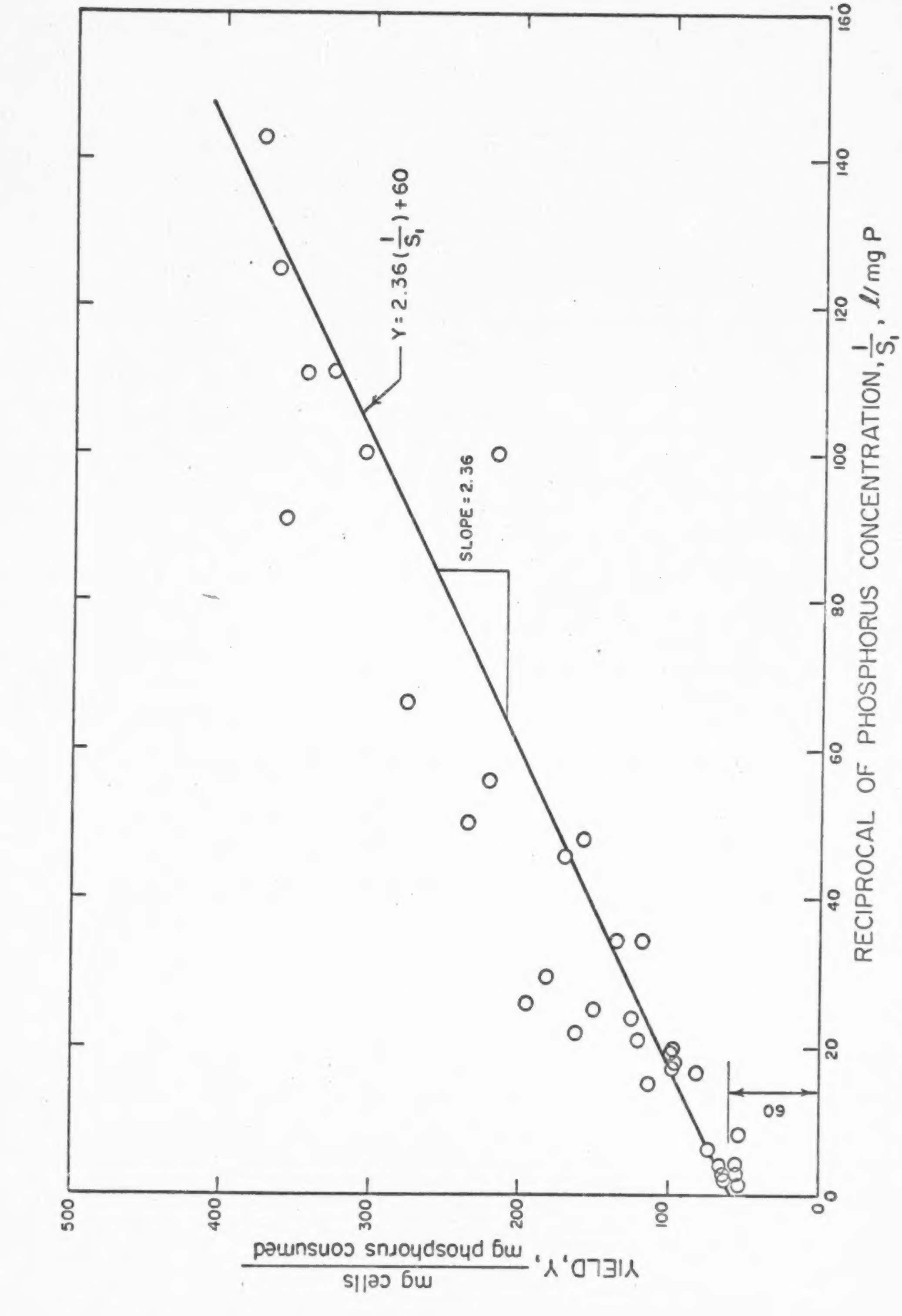

FIGURE 55. PLOT OF YIELD VS RECIPROCAL OF PHOSPHORUS CONCENTRATION

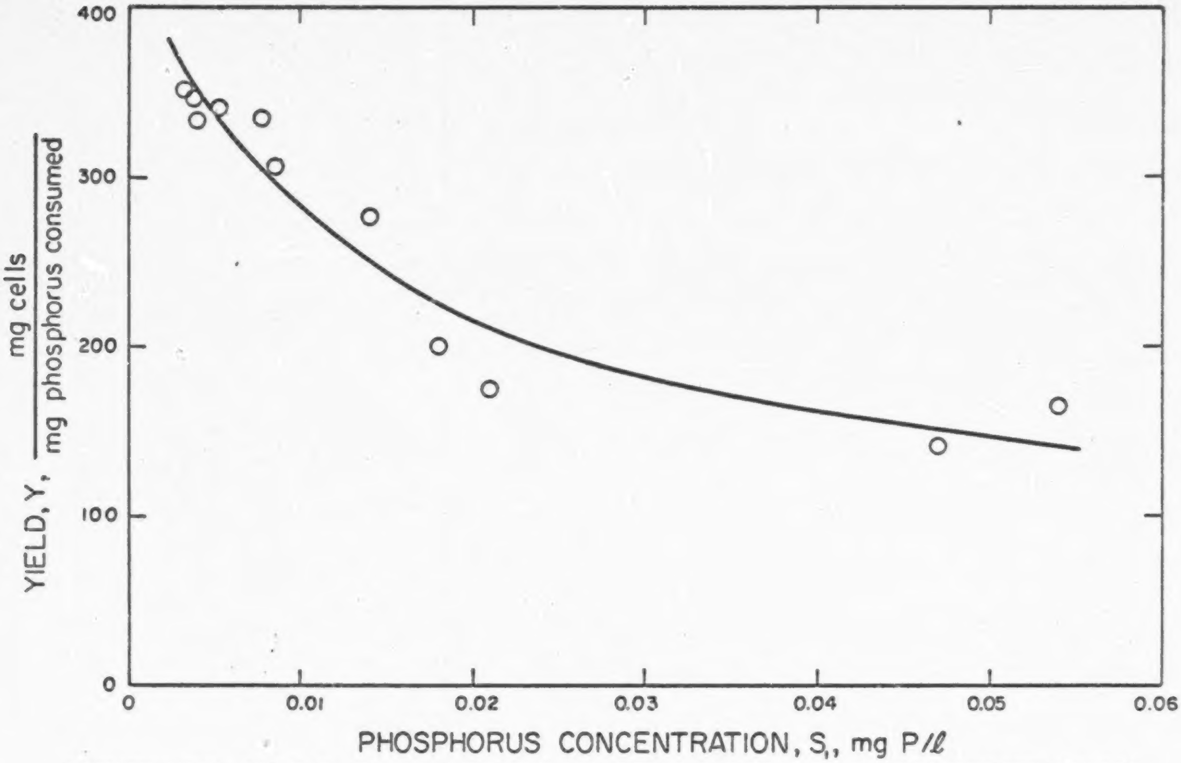

FIGURE 56. YIELD AS A FUNCTION OF PHOSPHORUS CONCENTRATION

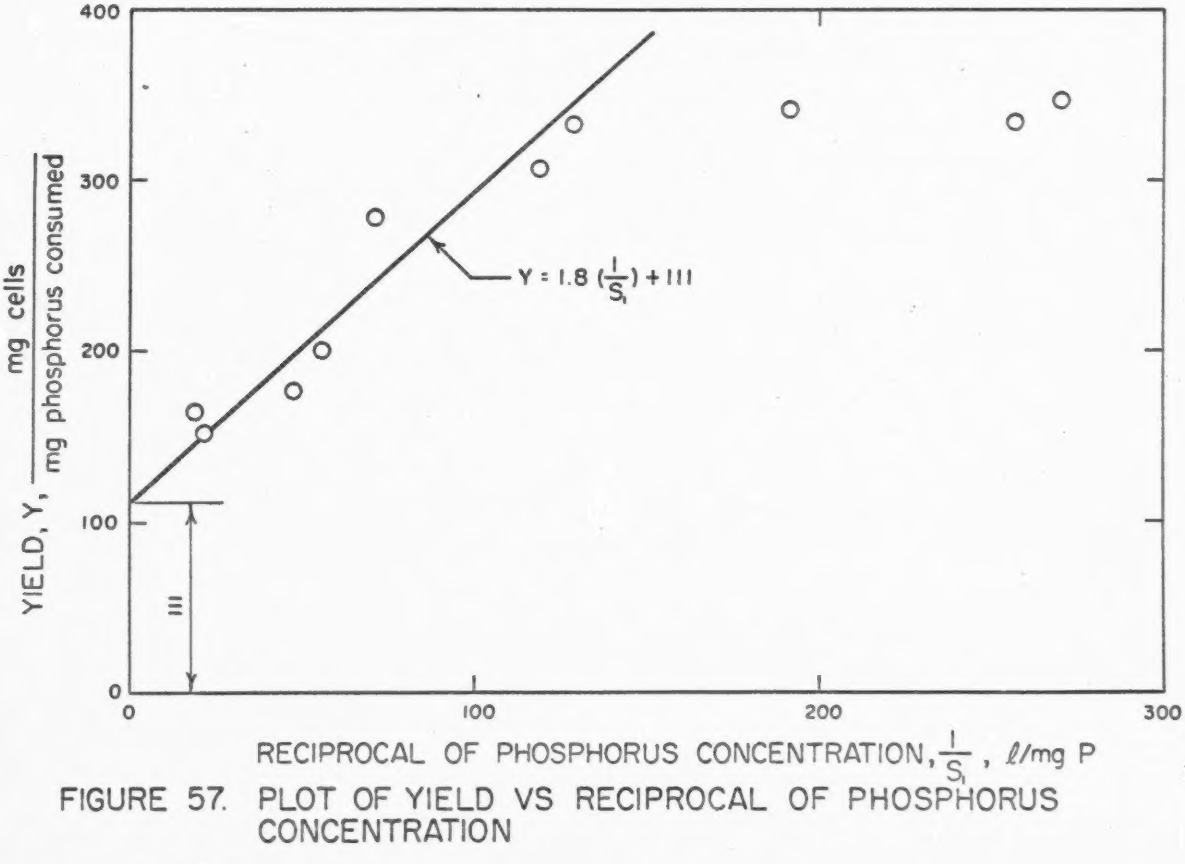

FIGURE 57. PLOT OF YIELD VS RECIPROCAL OF PHOSPHORUS CONCENTRATION

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Using estimates of C_y and Y_m determined from eye-fitted straight lines, predicted values of the algal concentration were calculated and compared with observed data. The results are shown in Tables XIV and XV for the 71105 strain and the Emerson strain, respectively. Predicted values of the algal concentration of the 71105 strain were also calculated using values of C_y and Y_m from the best-fit line by the least squares method but these appeared to be less satisfactory than those obtained with the values of C_y and Y_m obtained from the eye-fitted line.

From the data in Tables XIV and XV, it can be seen that fairly good estimates of the algal concentration can be obtained particulary at the higher dilution rates. An alternative procedure for predicting the cell concentrations at the higher dilution rates would be to assume a constant average value for the yield.

TABLE XIV

OBSERVED AND PREDICTED VALUES OF ALGAL CONCENTRATION

Expt. No.	Algal Concentration, x_1, mg/1		
	Observed	Predicted[a]	Predicted[b]
A-I-1	192	203	204
A-I-2	183	142	140
A-I-3	141	112	110
A-I-4	92	61	56
A-I-5	58	59	54
A-II-1	363	350	352
A-II-2	325	320	320
A-II-3	230	203	175
A-II-4	164	174	163
A-II-5	142	123	113
A-II-6	105	99	89
A-II-7	47	82	71
A-III-1	690	647	647
A-III-2	615	597	595
A-III-3	441	395	380
A-III-4	312	236	216
A-III-5	190	231	210
A-III-6	98	147	124
A-IV-1	760	1030	1028
A-IV-2	628	552	446
A-IV-3	465	556	491
A-IV-4	336	382	345
A-IV-5	245	292	254
A-IV-6	195	246	198
A-V-1	792	897	860
A-V-2	602	605	594
A-V-3	470	556	503
A-V-4	318	395	335
A-V-5	239	328	274
A-VI-1	830	1045	988
A-VI-2	685	841	738
A-VI-3	560	760	683
A-VI-4	435	536	452
A-VI-5	317	468	388

[a] Calculated using values of C_y and Y_m of line of best fit; $C_y = 2.24$, $Y_m = 73$.

[b] Calculated using values of C_y and Y_m of eye-fitted line; $C_y = 2.36$, $Y_m = 60$.

TABLE XV

OBSERVED AND PREDICTED VALUES OF
ALGAL CONCENTRATION

Expt. No.	Algal Concentration, x_1, mg/1	
	Observed	Predicted[a]
B-I-3	155	162
B-I-4	96	102
B-I-5	73	64
B-II-3	324	332
B-II-4	269	232
B-II-5	176	196
B-II-6	137	144

[a] Calculated using values of C_y and Y_m of eye-fitted line; $C_y = 1.8$, $Y_m = 111$.

CHAPTER V

CONCLUSIONS

The following conclusions were drawn from the results of this study:

1. When phosphorus is a growth-limiting nutrient, algal growth can be described by the Monod growth model which is written as follows:

$$\mu = \frac{\hat{\mu} S_1}{K_s + S_1}$$

where

μ = specific growth rate, time^{-1}

$\hat{\mu}$ = maximum specific growth rate, time^{-1}

S_1 = concentration of limiting nutrient, mass/volume

K_s = a 'saturation constant' numerically equal to the nutrient concentration at which the specific growth rate is one-half the maximum value, i.e., $\mu = \hat{\mu}/2$, mass/volume

2. The maximum specific growth rate, $\hat{\mu}$, of C. pyrenoidosa 71105, a high temperature strain, with the culturing conditions used is 4.19 day^{-1}. For the low temperature strain of C. pyrenoidosa, the Emerson strain, the maximum specific growth rate was found to be about half of that of the strain 71105. The value of the saturation constant, K_s,

with increasing dilution rate and also with increasing feed phosphorus concentration for any dilution rate. The results of the study showed that algae, specifically the 71105 and Emerson strains of *Chlorella pyrenoidosa*, can grow with as little as less than 0.3 per cent phosphorus in the cell mass.

7. For the 71105 strain of *Chlorella pyrenoidosa*, with low influent phosphorus levels (<5 mg P/1), the algal production rate, P, varies but little with the dilution rate and with equilibrium algal concentration. With higher feed phosphorus concentration, algal production increases to a maximum with increase in the dilution rate and then declines. The optimum dilution rate for algal production appears to be 3 day^{-1}. For a given dilution rate, algal production is proportional to the feed phosphorus concentration, S_0, up to some value of S_0 and then levels off with further increase in S_0. For the Emerson strain, high dilution rate or low culture concentration are more favorable for algal production.
8. Algal growth characteristics are significantly affected by the pH and temperature of the culture. Cell concentration and cell yield are lower, and the phosphorus content per unit cell mass higher, under unfavorable conditions of pH or temperature.
9. High degrees of removal (better than 85 per cent) of moderate concentrations of phosphorus (≤ 7 mg P per liter) can be accomplished by continuous algal cultures.

is of the order of less than 0.1 mg P/l for both strains.

3. The algal cell concentration at equilibrium varies inversely with the dilution rate. At a given dilution rate, the equilibrium concentration is also approximately proportional to the feed phosphorus concentration at low levels of the feed phosphorus.

4. The yield of cells with respect to phosphorus is not a constant but declines with increasing dilution rate and with increasing feed phosphorus level.

5. The cell yield declines, but approaches a constant value, with increasing residual phosphorus concentration in the medium, S_1. Within the limits 0.007 and 0.84 mg P/l of S_1, estimates of the cell yield may be obtained from the following relationship:

$$Y = C_y\left(\frac{1}{S_1}\right) + Y_m$$

where

Y = the cell yield, mg cells per mg of phosphorus consumed

S_1 = residual phosphorus concentration in the medium, mg P/l

C_y = the slope of a straight-line plot of observed values of Y against corresponding values of $1/S_1$.

Y_m = the y-intercept of the straight-line plot, corresponds to the minimum yield.

6. The phosphorus content of the algal cell mass increases

GLOSSARY

A	Specific maintenance rate, time^{-1}
B	Growth parameter
C, C_y	Constants
D	Dilution rate or volumetric flow rate of substrate per unit volume of culture, time^{-1}
F	Volumetric flow rate, volume/time
k_d	Specific decay rate, time^{-1}
K	Specific transfer rate, time^{-1}
K_A	Saturation constant equal to the concentration of limiting nutrient at which the specific growth rate is 0.632 of the maximum specific growth rate
K_g	Growth coefficient, time^{-1}
K_m	Maintenance coefficient
K_s	Saturation constant equal to the concentration of limiting nutrient at which the specific growth rate is one-half of the maximum specific growth rate.
n	Any number
P	Algal production rate, mass per unit volume per unit time
R	Respiration coefficient, time^{-1}
S	Concentration of limiting nutrient, mass/volume
S_0	Concentration of limiting nutrient in the feed to a continuous culture, mass/volume
S_1	Concentration of limiting nutrient in the culture and in the effluent, mass/volume
t	Time
t_d	Organism doubling time

T	Temperature, °C.
V	Culture volume
x	Concentration of organisms, mass/volume
x_0	Concentration of organisms in the feed to a continuous culture, mass/volume
x_1	Concentration of organisms in the culture and in the effluent, mass/volume
Y	Cell yield or yield factor, weight of cells produced per unit weight of nutrient consumed
$\hat{Y}$	Maximum yield factor
Y_m	Minimum yield factor
Θ	Detention period or hydraulic residence time, time
μ	Specific growth rate, time^{-1}
$\hat{\mu}$	Maximum specific growth rate, time^{-1}

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